Is a Peruvian mother's new pregnancy associated with changes in the dietary intakes of her breastfeeding child?

Allison Verney

School of Dietetics and Human Nutrition
McGll University, Montreal

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Abstract

Childhood malnutrition is attributed partly to inadequate feeding practices. This study examined the association of a pregnancy-breastfeeding overlap on dietary intakes of 89 children (10-36 mo) living in Lima, Peru. Their mothers were either not pregnant (NP; n=27), in early, 1st or 2nd trimester, pregnancy (EP; n=27), or in late, 3rd trimester, pregnancy (LP; n=35). Six-hour breast milk and complementary food intakes were measured between 9 AM- 3 PM and anthropometry, socioeconomic, and demographic data were collected. An overlap was associated with decreased breast milk intake in both the EP and LP groups compared to the NP group, however, there were no group differences in 6-hr total energy intake per kilogram of body weight after controlling for confounders. Complementary foods provide the vast majority of the diet and therefore, assuring their quality is key for child nutrition education.

Résumé

Dans les bas quartiers de Lima, au Pérou, la malnutrition chez les enfants est en partie attribuée à des pratiques alimentaires inadéquates. L'objectif de cette étude était de déterminer l'association entre le chevauchement grossesse-allaitement chez les femmes péruviennes et les apports alimentaires de leurs enfants. Au total, 54 dyades mères-bébés ont été recrutées. Certaines mères n'étaient pas enceintes (n=27) tandis que le reste se trouvait dans le premier ou deuxième trimestre de grossesse (n=27). Les apports alimentaires des enfants ont été observés pendant une période de 6 heures, de 9h à 15h. Le lait maternel et les aliments de complément ont été pesés grâce à des tests standard. Les données anthropométriques, socioéconomiques et démographiques ont également été collectées. Le chevauchement de l'allaitement et d'une nouvelle grossesse a été associé à une diminution de l'apport en lait maternel mais à une augmentation de l'apport énergétique total, après avoir pris en compte les variables confondantes. Sans égard à la décision de la mère de continuer à allaiter pendant sa grossesse, ces résultats démontrent que les aliments de complément sont majoritaires dans la diète. Ainsi, il est essentiel d'inclure des notions de qualité de ces aliments dans l'éducation nutritionnelle visant les enfants de cet âge.

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List of Abbreviations

Abbreviation	Full Name
AAFP	American Academy of Family Physicians
ANOVA	One-way analysis of variance
ASF	Animal source food
BMI	Body Mass Index
CI	Confidence Intervals
d	Day
EP	Early pregnancy
EsSalud	Portal del Seguro Social del Perú
g	Gram
HAZ	Height-for-age Z score
Kcal	Kilocalories
Kg	Kilograms
kJ	Kilojoules
IGF-I	Insulin Growth Factor
IIN	Instituto de Investigación Nutricional
LP	Late pregnancy
mg	Milligrams
mL	Milliliters
mo	Months
NP	Non-pregnant
OR	Odds Ratio
PAHO	Pan American Health Organization
PEN	Peruvian Nuevo Sol

Abbreviation	**Full Name (cont'd)**
SJL	San Juan de Lurigancho
SD	Standard deviation
UNICEF	United Nations Children Fund
USDA	United Stated Department of Agriculture
WAZ	Weight-for-age Z score
WHO	World Health Organization
WHZ	Weight-for-height Z score
y	Year

1 INTRODUCTION

1.1 Brief overview

Malnutrition in the developing world continues to have a debilitating effect on many infants and young children. It is estimated that globally 35% of child deaths before the age of 5 years are nutrition-related[1]. The United Nations Children Fund's (UNICEF) conceptual framework for determinants of nutritional status (Figure 1.1) explains that in addition to household food security and access to health services and healthy environments, care for children and women are also needed for child survival, growth, and development, all of which encompass child nutritional status[2, 3].

As children age, the contribution that breast milk makes to total energy intake decreases and, therefore, energy from complementary foods becomes more important. Choosing adequate, safe and appropriate complementary foods is essential in preventing childhood malnutrition; however, the choices of complementary foods may be inadequate and lead to growth failure.

There are many different determinants that affect the dietary intake of infants and young children but the effect of mothers' pregnancy status during breastfeeding has limited information. An overlap of pregnancy and lactation may influence a child's diet and nutritional status by changing feeding practices, which may be due to an interaction of hormones and maternal feeding patterns that lower breast milk intake and alter complementary foods. Although this lactation-pregnancy overlap is more common than expected, only a few documents have reported on its consequences and, so far, only in the mother and newborn[4-6]. Findings from this study may provide important insight into the effect of the mother's pregnancy status on the dietary intake and nutritional status of the child and may guide future efforts to promote effective recommendations on breastfeeding and complementary feeding.

1.2 Study rationale

Inadequate feeding practices, including breastfeeding and complementary feeding, are important determinants of malnutrition and morbidity in infants and young children. In Peru, breastfeeding is widespread and even extends into the second year of life; however, studies from Peru and other low and middle-income countries have reported that complementary feeding habits are often poor. This has resulted in growth failure in young children since they are not meeting energy requirements as recommended by the Pan American Health Organization[7].

Dairy research has demonstrated that when milking and pregnancy overlap, there is a decrease in milk production[8]; however, there is little research on this same overlap in humans. In countries where breastfeeding in the second year of life is common, breast milk may contribute to about one third of total energy intake[7]; if milk production decreases in humans as well, mothers need to compensate with an adequate amount of complementary food. Adequate complementary feeding is essential for growth and development of young children and, if not provided, the dietary intake of children may be inadequate, which in turn, may affect their nutritional status. A study conducted in a poor community of Lima, Peru, demonstrated that this lactation/pregnancy overlap continued into the first and second trimester of pregnancy in 60% of mothers who had a child under the age of four years[9]. This research project adds to our limited knowledge about the effects of this overlap and focuses attention on child feeding practices.

1.3 Overall study aim

The purpose of this study was to determine the association of concurrent lactation and early pregnancy on the dietary intakes of Peruvian infants and young children under the age of 36 months.

1.4 Objective and hypothesis

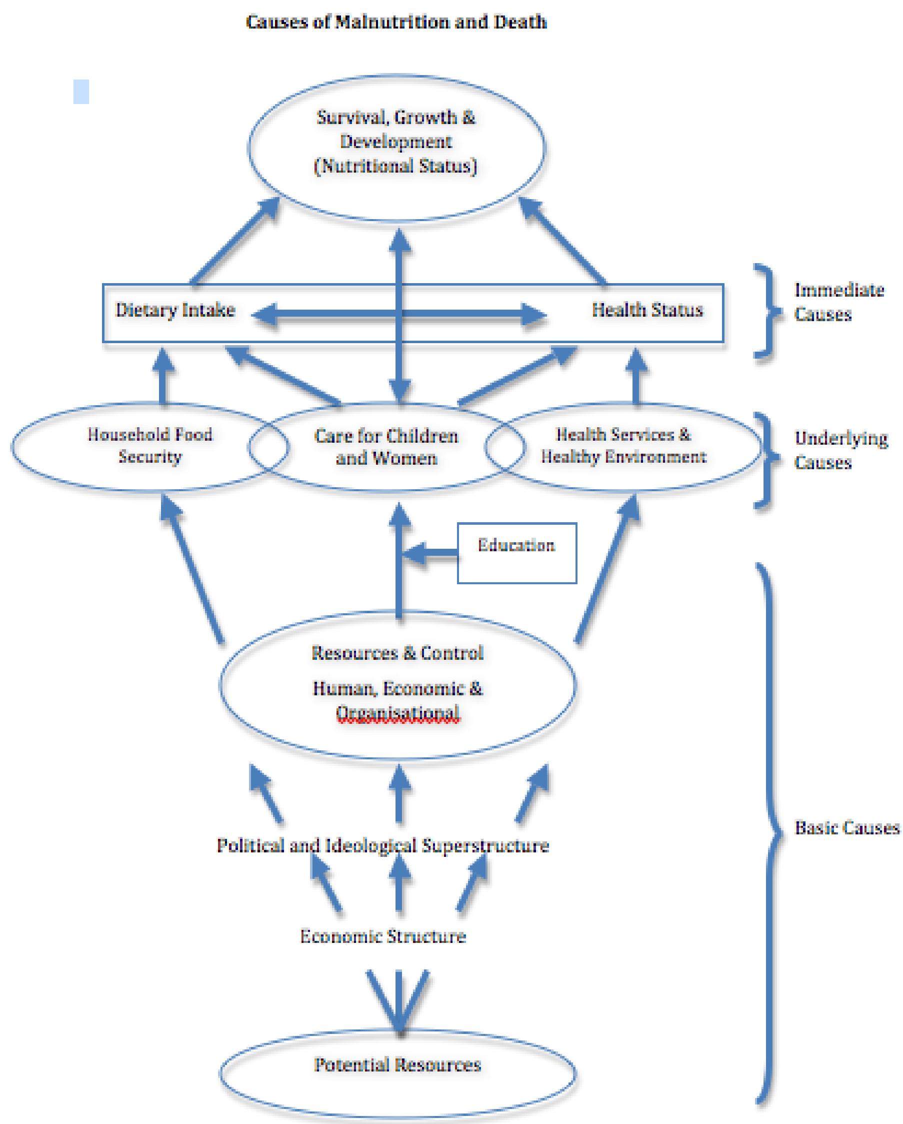
To compare the dietary intake of breastfed children under 36 months of age of mothers who are non-pregnant or in their 1st or 2nd trimester of pregnancy.

a. It is hypothesized that a breastfeeding child of a woman who is in the 1^{st} or 2^{nd} trimester of pregnancy would have a lower total energy intake than a breastfeeding child of a non-pregnant mother.

1.5 Explanation of conceptual framework

The conceptual framework underlying this study is a modification of the UNICEF framework for the causes of child malnutrition and the UNICEF framework of the state of the world's children, in addition to the extended model of care presented by Smith and Haddad [2, 3, 10]. This framework incorporates both the biological and socioeconomic causes that influence child survival, growth, and development at both the micro and macro levels. As explained by this framework, a child's nutritional status is the result of his/her dietary intake and health status, and dietary intake is the result of access to food and care for children and women. This conceptual framework recognizes that human and environmental resources, in addition to economic systems and political factors, are basic causes that contribute to nutritional status.

Figure 1.1 Conceptual framework as adapted from the UNICEF framework for the causes of child malnutrition and the UNICEF state of the world's children [2, 10].



2 LITERATURE REVIEW

2.1 Nutritional status of infants and young children

Anthropometric indicators of nutritional status demonstrate that malnutrition is prevalent among Peruvian infants and young children. Nutritional status can be assessed by using three indicators: weight-for-height (<-2 SD (standard deviations) is considered wasted), weight-for-age (<-2 SD is considered underweight), and height-for-age (<-2 SD is considered stunted)[1]. In Peru, wasting and underweight prevalences are low (0.6% and 4.2%, respectively); however, population surveys show that the overall stunting prevalence for children under 5 years is 23.8%, with the highest prevalence (29.8%) found in children 18-23 months of age[11]. Stunting reflects the cumulative effects of (i) inadequate dietary quality (micronutrients), (ii) repeated episodes of an illness (e.g., diarrhea, respiratory infections), or (iii) the presence of both simultaneously[12, 13]. Stunting may be indicative of chronic nutrient inadequacies, which is consistent with the documented high levels of certain micronutrient deficiencies seen in Peru. Anemia affects 60.3% of toddlers 12-17 months old, 49.2% of 18-23 months old, 34% of 24-35 months old, and 25.6% of 36-47 months old, which are among the highest prevalences in South America[11]. In Peru, more than half of anemia cases are caused by low iron intake and young children in particular are at a high risk of iron deficiency anemia since, in addition to small iron reserves, they are in a period of rapid growth[14, 15]. Additionally, there are reasons to suspect that children with a low socio-economic status in peri-urban Lima may be zinc-deficient since, in combination with their inadequate dietary intake, there are high prevalences of diarrhea, which causes excess fecal losses of zinc, and therefore low plasma zinc concentrations[14, 16].

2.1.1 Growth faltering

Growth faltering, often the result of malnutrition, is highly prevalent in developing countries and is associated with many adverse outcomes throughout the lifespan such as lower levels of schooling, lower human capital, and illness due to chronic diseases. Inadequate energy intake and frequent infections are well-known causes of growth faltering but the specific mechanisms of inadequate nutrient intake on growth faltering in young children are not entirely known; it is challenging to interpret the association between energy intake and linear growth since, for the most part, when energy intake is low, the intake of nutrients is low as well[17]. Height is the result of the interaction between a genetic component and both micro- and macronutrient availability throughout growth. During longitudinal growth, chondrocytes develop through a series of well-defined stages, which are characterized by changes in proliferation, shape, size, synthesis and deposition of extracellular matrix components[18]. The addition of new cells to the growth plate of the bone and hypertrophy result in the expansion of the growth plate[18]. Nutrition has been seen to play an important role in linear growth. The mechanism by which zinc plays a role in growth faltering may be through its role in decreasing both insulin-like growth factor-I (IGF-I) plasma concentration and growth hormone receptors, which are important components of proliferation in bone chondrocytes and synthesis of collagen and proteoglycans[19]. Evidence from animal studies has indicated that energy and protein restrictions reduce IGF-I plasma concentration but when energy and protein levels are replenished, IGF-I returns to normal[20]. One study produced a three-day fast in rats where they found a reduction in IGF-I levels by 18% compared to the fed controls, but with re-feeding of the fasted rats, IGF-I levels were normalized[21]. In general, animal source foods contain higher concentrations of protein and bioavailable micronutrients than plant food that may limit growth (such as calcium, zinc, iron and vitamin A) and micronutrients in animal source foods are generally more readily available than micronutrients from plant foods. Another study, conducted in Mexico, used longitudinal data from 67 toddlers 18-30 months of age to evaluate growth rate and predictors of attained size[22]. Size at 30 months and growth rates were unrelated to energy or protein intakes during the previous year but were positively related to consumption of animal-origin foods ($p < 0.01$).

Marquis and colleagues analyzed data from 107 breast-fed and weaned Peruvian toddlers to determine if breast milk or animal source foods contributed to improved linear growth between the ages of 12 and 15 months[23]. Intake of animal source foods was associated with an increase in linear growth of 0.4 cm in a 3-month period among infants who were either breastfed infrequently or weaned. However, neither of these cross-sectional studies were able to prove that there is a direct effect of animal source food intake on growth; a randomized control trial using animal source complementary food supplements is necessary.

In addition to animal source foods, energy intakes have been seen to be associated with linear growth. Gopalan and colleagues conducted a study to see the effect of different energy levels on the growth of 415 children between the ages of 1 and 5 years[24]. The supplements were provided over a period of 14 months with 276 feeding days. The children were divided into the two groups: an experimental group that received supplements (n=309) and a comparison group that did not receive supplements (n=109). The food supplement consisted of wheat flour, sugar, and edible oil and provided 310 kcal and 3 g protein. The experimental group showed increases in both weight and height during the study compared to the children who did not receive the supplement ($p<0.03$). Results of clinical assessment at the end of the 14 months showed that there was a considerable reduction in the prevalence of signs of protein-energy malnutrition in the supplemented group.

Other studies have looked solely at the growth patterns of infants and young children. Shrimpton *et al*. re-analyzed datasets from 39 different surveys and studied the timing and patterns of growth faltering by calculating the mean weight-for-age, height-for-age, and weight-for-height z scores[25]. The growth profile demonstrated that the process of stunting begins at birth and continues during the first three years of life, after which no recovery from stunting was observed. Victora *et al*. reassessed the study using the new WHO Growth Standards and included other countries datasets[26]. Datasets from 54 countries with national anthropometric surveys showed that growth faltering occurred mostly between 3 and 24 months of age and was more pronounced in children from low-and middle-income countries. In general, weight-for-age (WAZ) starts close to the WHO standards at birth and then falters slightly until it reaches a z score around -1 at 24 months and then remains stable thereafter. Length/height-for-age (HAZ) also starts close to the WHO standards at birth but falters considerably until 24 months and then begins to increase slightly after. The Peruvian data from this study show a different trend to those seen worldwide. HAZ at 1-month was -0.43, at 12-months was -1.18, and at 24 months, -1.52. Unlike the trends worldwide, in Peru, the HAZ at 36-month was even worse at -1.87 but then slightly improved thereafter. WAZ in Peru did not follow the same worldwide trend; WAZ at 1-month was 0.21 and then faltered slightly until -0.42 at 24-months; however, it continued to decline until 36 months where the WAZ was -0.85. It began to stabilize at 59-months and then began to improve.

2.2 **Dietary intakes of infants and young children**

Young children require special attention since their dietary requirements are rapidly increasing. During this transition period, a toddler's breast milk is being replaced by common foods as eaten by the family; at one year of age, a child is able to consume the same foods as would be present in an adult's diversified diet[27].

Complementary feeding is defined by PAHO/WHO as the additional nutrition when breast milk alone is no longer able to meet the nutritional requirements of children, and therefore other foods and liquids are needed to augment breast milk [7]. The target age range for complementary feeding is between 6 and 24 months; however breastfeeding may continue into the third year of life and beyond. Complementary foods should contain a variety of meat, poultry, fish, or eggs; vitamin A-rich fruits and vegetables; and an adequate amount of fat. Dietary fats provide children with essential fatty acids, energy, and allow absorption of fat-soluble vitamins. Fats may also increase palatability of foods, which promotes greater total energy intake[28]. There is little information available on the optimal levels of total dietary fat for young children, but the range of 30-45% has been suggested[27, 29]. If a minimal amount of fat is provided to ensure an adequate supply of essential fatty acids, and that energy density is within an acceptable range, some researchers believe that fat intake should not be restricted for children less than 2 years of age[30].

2.2.1 Peruvian complementary feeding

Marquis and colleagues examined the patterns of complementary feeding in 20 Peruvian toddlers 12-18 months of age living in a peri-urban community of Lima; 20 were breastfed toddlers and 10 were non-breastfed toddlers[31]. They found the average energy density of Peruvian complementary food to be 1.6 ± 0.1 kcal/g with 71.9 ± 15.1% of this complementary food being consumed in the daytime (7 am to 7 pm). Energy intake from complementary foods was 488.7 ± 194.8 kcal/d and total energy per kilogram of body weight was 85.6 ± 23.2 kcal/kg. This same study found that daytime intake of breastfed toddlers had more energy from snacks and less from main dishes than non-breastfed toddlers ($p<0.01$). Additionally, almost half of the breastfed toddlers' energy was from savory snacks (30%) and sweets (19%). Another study conducted in the same community analyzed the consumption of food and nutrients by infants[13]. They found that most of the children consumed roots and tubers (mainly potatoes and sweet potatoes) by one year of age but only 51% of children were consuming separated fats and oils and only extremely small quantities were eaten. Soup was the most typical complementary food prepared in the community.

The types of complementary foods provided to young children are essential for growth. Zeitlin and Ahmed, in a study of 165 Bangladeshi mothers and breastfed infants and toddlers aged 5-23 months, found that height-for-age z-scores decreased between 12 and 18 months of age, with height-for-age z-scores being -1.9 and -2.6 at 12 months and 18 months, respectively[32]. During this period, they found that breastfeeding frequency declined while energy intake from complementary foods remained the same. This study suggests that if breast milk intakes decrease, then the amount and type of complementary foods become even more important for child growth. However, as seen in the complementary foods provided to Peruvian children, the energy intake from these complementary foods may be inadequate to meet the daily energy requirements if energy from breast milk decreases.

2.2.2 The Pan American Health Organization recommendations

The *PAHO guidelines for complementary feeding of breastfed children (2004)* recommends age-specific complementary food energy requirements by subtracting average breast milk intake from the total energy requirements[7]. The mean daily energy requirement for healthy, breastfed 12-23 mo-olds is 894 kcal. In developing countries, average breast milk intake of a healthy 12- to 23-mo-old child is estimated to be 346 kcal/d[33]. The appropriate quantity of complementary foods that would meet the energy needs for a child of 12-23 months is 378-515 g/d. Many children in low-income countries consume less than the recommended energy and nutrient requirements, even when breastfed[7]. This could be the result of illnesses such as diarrhea or respiratory infections since children affected by these illnesses have reduced appetites, malabsorption of nutrients due to intestinal hindrance and problems, and excess losses of nutrients[34-36].

2.3 Determinants of dietary intake of complementary foods

2.3.1 Energy density and feeding frequency

Proper feeding frequency and energy density of foods are two important determinants to help infants and young children meet their nutritional needs. However, with young children's limited stomach capacity and their increasing energy needs, this can sometimes be a challenge[37]. Additionally, if complementary feeding has a high energy density or occurs too frequently, breastfeeding frequency and duration has been seen to occur less often and for a shorter time[7].

In low-income countries, complementary foods tend to be low in energy density, which suggests that foods and meals should be provided more frequently throughout the day to meet the child's increasing energy demands. Islam *et al*. conducted a study with 18 healthy breastfed children 8-11 months of age who were fed porridge with different energy densities (0.5, 1.0, or 1.5 kcal/g) and with varying feeding frequencies (3, 4, or 5 times per day) during 9 randomly assigned 3-6 day dietary periods[38]. They found that total mean daily energy intake from complementary foods decreased by 18 % when energy density increased from 0.5 to 1.5 kcal/g ($p<0.001$), indicating an inverse relationship. Furthermore, the mean amount of total energy intake increased by 42% when feeding frequency increased from 3 to 5 meals/d, indicating that feeding frequency was positively associated with energy intake ($p<0.001$). However, breast milk intake decreased by 11 % and 8% with increased energy density ($p<0.001$) and feeding frequency ($p<0.04$), respectively, therefore caution must be taken when recommending the energy density of complementary foods to avoid displacing breast milk.

Brown *et al*. conducted a study to evaluate energy density by using nonfat additives from 1.67 to 6.28 kJ/g in 18 Peruvian children from 6 to 18 months who were recovering from severe malnutrition[37]. The results demonstrated that overall the energy intake was more than twice as great with the highest energy density foods compared with the lowest. It also demonstrated that when energy density was taken into account, the total daily consumption was about 16% greater when the number of meals was increased from three to four per day ($p< 0.001$) and 7% more when the feeding frequency rose from four to five per day ($p= 0.005$). However, this study was conducted in a controlled study setting where trained caregivers used standardized feeding procedures with the children, so it is yet to be seen if these results would be the same in natural conditions where the effect of the caregivers' behaviours is also considered.

2.3.2 Diet quality and diversity

Diet quality and diversity in many studies have been shown to be important for child nutrition and can be a predictor of nutritional status, as they are shown to correlate highly with micronutrient density adequacy[39-42]. Onyango *et al.* examined feeding behaviours among 154 rural western Kenyan toddlers aged 12-36 months and found that anthropometric values were consistently and positively associated with an increase in different foods consumed (WAZ $p=0.02$, HAZ $p=0.008$, WHZ $p=0.01$)[43]. They found that the partially breastfed toddlers who had lower dietary diversity had nutrient intakes from about 20% to 50% below the partially breastfed toddlers with high diversity. However, this study considered dietary diversity as the count of the number of different foods consumed and not necessarily of different food groups consumed, so although the foods may be diverse, the nutritional quality may not be.

When children are being introduced to various complementary foods, cereal-based gruels are often among the first to be introduced. Sometimes in resource-poor areas, children's diets continue to rely on this monotonous staple; however, these foods are often low in energy nutrient density [44]. Anderson *et al.* conducted a study to examine the feeding practices and the energy and nutrient intakes from complementary foods of two groups: breastfed and non-breastfed stunted Cambodian young children aged 12-42 months[45]. The study revealed a diet where meat, poultry, and fish provided less than 10% of their energy, which may have been associated with poor growth in both the breastfed and non-breastfed groups since other studies found that an increase in animal source foods has been positively associated with improved growth, especially in children who have low energy from breast milk[23, 45].

2.3.3 Maternal education

Maternal education has been shown to be associated with child nutritional status. Hendricks *et al.* used data from the United States in the 2002 Feeding Infants and Toddlers Study and conducted a descriptive analysis from the data obtained from this cohort study[46]. They found that mothers with a college education were significantly more likely than mothers without a college education to breastfeed a toddler at 12 months (OR 3.9, 95% CI 2.3-6.6) and to comply with complementary feeding recommendations (OR 2.0, 95% CI 1.5-2.7). This study did have slightly more middle- and high-income mothers than what is found in the national distributions of households' income, and therefore may not be an accurate representation of all mothers. Also, it was a cross-sectional study, which used only one 24-hour dietary recall instead of following the mothers and children over time. Multiple 24-hour dietary recalls may have provided more accurate information on infant and young children feeding practices.

A study in Nepal demonstrated that children 6-36 months of age (n=443) whose mother had more than 5 years of education were less likely to be stunted than children from less educated mothers (OR 0.57, 95% CI 0.37-0.89) [47]. Another study conducted in Benin and Mali found that in both countries, mothers with no education were more likely to have children with severe and moderate levels of anemia compared to mothers who had any education ($p < 0.001$) [48, 49]. Some studies have mixed results regarding the effect of maternal education on child's dietary intake. Ruel and Menon discovered that in Peru, child-feeding practices interacted with maternal education, although it was only approaching significance ($p=0.06$)[50]. This interaction suggested that child-feeding practices were positively associated with better nutritional status. However, this positive association was only among mothers who had some primary school education; mothers with any additional education than only primary school did not have children with better nutritional status than mothers who had no education.

2.3.4 Socioeconomic status

Higher socio-economic status is thought to improve nutritional status of children through increased food availability, improved hygienic living conditions and better access to health care. Many studies have shown that malnourished children are more likely to come from poorer households[51-53]. Hong and Mishra used information on 3235 children under 59 months of age to assess the associations of economic inequality and stunting[53]. The results indicated that children in the poorest 20% households were more than twice as likely to suffer from stunting as children in the richest 20% households (OR 2.54, 95% CI 1.91-3.39). On the other hand, a case-control study in Mexico examined differences between stunted and well-nourished children 6-23 months of age in rural and urban areas[54]. The study found that *per capita* family income per month was not significantly different between cases and controls in the rural area but was significantly different in urban areas with more stunted children coming from households with lower *per capita* family income per month than the well-nourished children (OR: 1.65, 95% CI 1.03-2.64). This suggests that low household income is not necessarily a predictor of malnutrition in children in rural areas but may be in urban areas.

2.3.5 Infantilization

Levy first identified the concept of infantilization in 1943 as one form of maternal over-protection[55]. He used the term literally meaning care given to a very young child. The concept of infantilization, or mothers' perception of their child's age, is also a determinant of dietary intake. One study conducted in Peruvian toddlers found that differences in feeding patterns between breastfed versus non-breastfed toddlers may be due to maternal perceptions of breastfed children as "still young" and therefore the mother continues to feed the child more as an infant by providing more soups and easily digestible foods rather than as a young child[31]. The study found that breastfed toddlers ate small amounts of foods and liquids other than breast milk throughout the day while non-breastfed toddlers received most of their energy from main Peruvian dishes. Breastfed toddlers received only 17% of their total energy from the main dish versus a weaned toddler who received 32% of their total energy from the main dish. Additionally, breastfed toddlers received 19% of their total energy from sweets versus 7% for the weaned toddlers. Considering the low density of nutrients found in sweets, this high percentage in breastfed children may be displacing energy from more nutritious foods. However, total energy intake was similar for the breastfed and weaned toddlers. This concept of infantilization has been seen in other areas of feeding and parenting: a mother may persistently breastfeed or bottle feed beyond the usual age of weaning; she may not provide foods eaten by the whole family or she may routinely assist with the child's feeding, dressing, and toileting, despite the child's ability to perform such tasks autonomously[56]. This concept of infantilization has not been well studied to date but it could have implications for toddlers' dietary intakes.

2.4 **Determinants of breastfeeding**

In most low-income countries, mothers continue to breastfeed their infant and young children well into the second year of life. In Peru, the prevalences of breastfeeding children 9-11 mo-old, 12-17 mo-old, 18-23 mo-old and 24-36 mo-old are 91.0%, 76.4%, 50.7%, and 20.6%, respectively[11].

There are many different determinants of breastfeeding such as child's age, maternal education and employment. As children age and begin to consume more foods and liquids other than breast milk, they become less dependent on breast milk as a form of energy and may breastfeed less often[7, 57, 58]. Maternal education is also associated with breastfeeding. It has been seen in low-income countries that higher maternal education is negatively associated with breastfeeding. One study in rural Bangladesh found that higher educated mothers were more likely to stop breastfeeding ($p<0.05$) and the children spend less time at the breast ($p<0.05$) than less educated mothers[59]. The greater tendency for educated mothers to work away from home than non-educated mothers may provide an explanation for why these mothers tend to have fewer breastfeeds or wean their child sooner. One study analyzing the breastfeeding patterns in Yemen, Egypt, Jordan and Tunisia found that the probability of still breastfeeding at 15 months was positively associated with women who do not work at all or who work at home ($p<0.01$)[60]. However, Butz concluded that it is inappropriate to assess the impact of working status on breastfeeding through oral reports since mothers may say work was the main reason for decreased or terminating breastfeeding, while there could be other factors that indirectly influence breastfeeding[61]. One factor may be the increased socioeconomic status of the household since it has been observed to be associated with decreased duration of lactation among Bangladeshi women ($p<0.02$)[59]. This could be due to increased economic wealth, where the mother is able to afford safe and adequate complementary foods, and begin to replace breast milk.

2.4.1 Milk production

It is normal for healthy infants to self-regulate breast milk intake to meet their needs but when the milk production is limited, it may lead to an insufficient human milk intake[62]. Milk production is affected by various factors including a mother's feeding practices, children's appetite, state of maternal health, and perceived insufficient milk production.

2.4.2 Mothers' feeding practices

Mothers' feeding practices, such as feeding frequency or duration per breastfeed, can affect milk production. In cows, it has long been known that the number of milkings within a 24-hour period influences milk production. One study by Amos and colleagues used 34 mature Holstein cows and assigned them randomly to groups of either twice or thrice daily milkings[63]. They found that multiparous and first-lactation cows milked thrice daily produced 18.5 and 25.2% more milk than their counter-parts who were milked twice daily. One study in Bangladesh found that children 12-17 months (n=47) and ≥ 18 months (n=61) who were at the breast for less than an hour and breastfed under five times in a 12-hour period compared to children of the same age who were at the breast for more than 90 minutes and breastfed more than eight times in a 12-hour period had differences of breast milk intake of 186 g and 157g, respectively[64]. This demonstrated that when frequency and duration increased so did the amount of breast milk consumed ($p<0.001$ and $p<0.001$, respectively).

2.4.3 Children's appetite

Children's appetite can decrease during illness. One study carried out among 56 Peruvian children 9-20 months of age found that decreased appetite associated with illness had a strong negative effect on both liquids other than breast milk ($p<0.000$) and complementary foods ($p=0.001$) but that these negative effects were not seen with breast milk[65]. This could be explained by severity of the illness, and feeding frequency. The continued breastfeeding during illness may indicate that the child continued to suck to satisfy thirst or that the appetite for breast milk or wanting to suck is regulated by a different mechanism than the appetite for other foods; however this mechanism is unknown[66]. PAHO recommends that a mother continues to breastfeed frequently throughout her child's illness[7].

2.4.4 Maternal health

Maternal health has been demonstrated to have an effect on milk production, however the results are inconsistent[67]. Some studies have found a correlation between maternal Body Mass Index (BMI) and milk fat and therefore energy levels; however, other studies find no such association[68-70]. Prentice *et al.* conducted an extensive review of the literature, using 41 databases with 1726 measurements, to see if BMI was a useful indicator to evaluate impaired milk production[71]. Maternal nutritional status was not found to have a relationship with milk production even when analyzed according to the mean BMI of different populations and even when BMI was classified as underweight. There are conclusive data, however, demonstrating that the composition of milk changes from mother to mother, day to day, and with the mother's diet[72, 73]. Brown *et al.* found that changes in nutritional status within individual women were significantly related to the amount of milk produced along with the milk composition; within-woman increases in triceps skin-fold thickness were associated with increases in milk fat and energy concentrations ($p < 0.01$) and within-woman body weight increases were associated with increases in the yield of milk and other major nutrients *(p* < 0.01)[68].

2.4.5 Insufficient milk production

Regardless of maternal nutritional status, low milk production can be seen among some women due to the perception of having insufficient milk. Gussler and Breisemeister were the first to write about, what they termed, "insufficient milk syndrome"[74]. They provided the first bio-cultural explanation of the phenomenon arguing that, in urban settings, rather than being solely a culturally appropriate excuse to discontinue breastfeeding, milk insufficiency is associated with extended periods of separation between mothers and infants, and planned, rather than being *ad lib*. More recent studies of milk insufficiency have concluded that cultural practices, psychosocial factors, and breastfeeding behaviour are directly linked to perceived milk insufficiency. One study in Mexico interviewed 165 healthy Mexican mothers attending a public hospital about breastfeeding and found that 80% of the women reported perceived insufficient milk at one point during the study period[75]. It was found that lack of confidence in breastfeeding ($p=0.02$) and delayed onset of milk production ($p=0.02$) were positively associated, and maternal education ($p=0.01$) was negatively associated, with perceived insufficient milk. Determinants of lactation success changed as the child grew older, but socio-cultural and bio-cultural determinants were always more important than biological factors such as birth weight, sex, and maternal weight. Another study in rural Bangladesh reported that 255 mothers stopped breastfeeding their child because of perceived insufficient milk production; 59 % of whom stopped breastfeeding once they became pregnant[59]. Behavioural studies in the developing world have indicated that pregnancy is a common reason for changing breastfeeding practices. Hillervik-Lindquist and colleagues conducted the only longitudinal study that used test-weighing as a method to evaluate perceived milk insufficiency[76]. Fifty-one educated Swedish mothers were given scales and asked to measure their infants' weight and 24-hour milk intake at eight different time points. Twenty-eight mothers experienced milk insufficiency at some point. The authors concluded that while there was no significant difference in measured milk intake during, and one week after, the "perceived milk insufficiency", infants' weight-for-age z scores were significantly lower compared to infants from mothers who did not perceive milk insufficiency.

2.4.6 Extended breastfeeding

WHO has recommended the continuation of breastfeeding until 2 years of age or beyond[77, 78]. In many countries, breast milk constitutes a major share of the overall fat, protein and energy intake of children even up to the age of 3 years, and thus contributes significantly to the nutritional well-being of the child. One study found that the 24-hour estimates of human milk intake in breastfed 12-18 mo-old toddlers living in peri-urban communities of Lima, Peru were 488.7± 194.8 g, which corresponded to about 39% of their total energy intake[31]. In Bangladesh, children 24-30 months of age consumed 368 ± 17 g of breast milk, which corresponded to about 31% of their total energy intake[79]. In New Guinea, one study found that breast milk contributed to 17-54% of the energy consumed at 12-23 months[80].

It is evident that breastfeeding continues in the second year of life in many countries and can continue to provide a large contribution to total energy intake. However, the effects of extended breastfeeding on child nutritional status have been debated among researchers. One study observing 4515 Senegalese toddlers collected information to determine why mothers extended breastfeeding[81]. The median duration of breastfeeding was longer for toddlers with length-for-age z-scores less than 2 SD and was shortest for toddlers with a length-for-age z-score above zero SD (25 months and 22.7 months), respectively; $p<0.0001$). At 24 months, the probability of still being breastfed was nearly 71% for children with a length-for-age lower than -2 SD z-scores and 37.5% for those who had a length-for-age z-score above zero SD, demonstrating that mothers extended breastfeeding when the child had a poorer nutritional status. Similarly, Victora *et* *al*. observed 802 children aged 12 to 35.9 months in southern Brazil and found that children being breastfed had a significantly higher prevalence of low weight-for-length than those who were already weaned ($p = 0.02$)[82]. This association was still apparent even after controlling for confounding variables including child's age, sex, birth order, income, maternal education, employment status of the family head, district of residence, and ethnic background.

Marquis and colleagues conducted a study in a peri-urban community of Lima, Peru to evaluate the reversible nature of child-feeding decisions, particularly breastfeeding, weaning, and re-lactation patterns[83]. Data from 32 mothers (21 lactating mothers and 11 mothers who had recently weaned their children) were used in the analysis. The study found that poor child health, poor eating habits, and slow motor and language development were often seen as reasons to extend breastfeeding so as not to increase the child's health risk. Mothers weaned their children only when they were considered to be healthy and sufficiently "big". Another study examined the associations of breastfeeding and stunting in 134 Peruvian toddlers between 12-15 months of age[84]. They found a significant ($p < 0.01$) interaction of breastfeeding, diarrhea incidence, and complementary food intake on the child's length at 15 months. When the toddler's dietary intake was low and diarrhea morbidity was high, increased breastfeeding was associated with a 1.0 cm decrease in length gain. The negative association between breastfeeding and linear growth may have reflected a reverse causality: a phenomenon whereby the mother's decision to continue breastfeeding is influenced by the child's poor nutritional status[81, 85]. It may be that extended breastfeeding may not lead to poor growth; instead, it could be children's poor growth and health that led to increased duration of breastfeeding.

2.4.7 Advantages for extended breastfeeding

Healthy breastfed children between the ages of 12-24 months, assuming an average breast milk intake, consume about one-third of their total energy intake from breast milk. This contribution of energy from breast milk can ameliorate a low quality diet of complementary foods, especially for protein, fat and micronutrients such as vitamin A, calcium and riboflavin[7, 86]. Breast milk is a more abundant source of fat (40-55%) than most complementary foods (30-45%), which is why fat intake usually decreases with age as the contribution of breast milk to total dietary energy declines; however, children in low-income countries are often not even meeting the lower range of fat in complementary foods[33]. Breastfeeding beyond the first year of life has been demonstrated to help prevent xerophthalmia, which is caused by vitamin A deficiency. Mahalanabis conducted a case-control study in a diarrhea treatment centre in Bangladesh to determine the effect of breastfeeding on the risk of xerophthalmia[87]. He studied 2687 children aged 6-36 months; 66 of the children were cases of xerophthalmia and the other 2621 children did not have symptoms of vitamin A deficiency and served as controls. Approximately 50% of the children aged 24-35 months in the study were still being breastfed. The study found that breastfeeding was associated with a 74% reduction in the risk of xerophthalmia caused by vitamin A deficiency in children under 24 months (OR 0.26, 95% CI 0.14 - 0.49) and a 65% reduction in children between 24-36 months (OR 0.35, 95% CI 0.35- 0.86).

In addition to prevention of xerophthalmia, breastfeeding has also been found to be positively associated with linear growth. For example, Marquis and colleagues found that, among toddlers 12-15 months of age, breastfeeding had a positive association with linear growth when they had a low intake of animal source foods compared to weaned toddlers ($p<0.05$)[23]. There was a 0.5 cm difference in linear growth over 3 months between weaned toddlers and children who consumed the average number of feedings of breast milk.

2.5 Pregnancy/breastfeeding overlap

Many determinants of infants and young children’s dietary intake have been thoroughly researched, however, the determinant of a mother’s pregnancy status during breastfeeding has limited information available. There are cultural taboos against breastfeeding during pregnancy throughout the world and only recently has more research reviewed this overlap[88-90]. One study used cross-sectional data from Bangladesh and discovered that 45% of breastfeeding women who became pregnant continued to breastfeed through the sixth month of pregnancy[59]. Merchant *et al.* conducted a study with 504 rural Guatemalan women and discovered that 50.2% were breastfeeding during pregnancy[91]. When this overlap occurred, 41.4% continued into the second trimester and 3.2% continued into the third trimester. A study with Peruvian women showed that 60% of pregnant women who lived with a child under the age of 4 years were also breastfeeding this child in the first and second trimesters of the new pregnancy; 10% of women continued to breastfeed into the third trimester[9].

Although this practice seems to be prevalent, there are only a few reports and books that even mention it. The American Academy of Family Physicians (AAFP) wrote a policy statement in 2008, which included the breastfeeding-pregnancy overlap[6]. The AAFP paper commented on the overlap being practiced in the United States and that weaning a child would increase the risk of illness since extended breastfeeding provides continued immune protection and a sustainable food source. Lawrence suggested that tandem nursing, breastfeeding the toddler and the newborn simultaneously, provides an easier transition psychologically for the child[4]. Self help books have also encouraged breastfeeding through pregnancy[5]. However, evidence from human and animal research demonstrates that a pregnancy-breastfeeding overlap may in fact have negative effects on the young child.

2.5.1 Animal research on the lactation consequences of the overlap

Although the consequences of the overlap have not been researched in children, studies on the change in milk yield and milk composition have been seen in animals. Olori and colleagues evaluated effects of gestational stage on daily milk production and composition using 325 Holstein Friesian cows[8]. They found that gestational stage had a significant effect ($p < 0.05$) on milk, fat, protein, and lactose. The daily milk yield declined due to pregnancy from month one of gestation and continued until parturition; however, the significant decrease ($p < 0.05$) in milk yield did not begin until the 5th month of pregnancy when daily milk production of pregnant cows was over half a kilogram less than the yield of non-pregnant contemporaries. The average daily milk yield of cows that were 8 months pregnant was about 3.0 kg, corresponding to about 11% below the production level of non-pregnant cows. Coulon *et al*. found similar results: at the 29th week of pregnancy, the milk yield was 2.4 and 3.6 kg/d lower in the low-medium producing pregnant cows and the high producing pregnant cows, respectively, versus the non-pregnant cows[92]. Bar-Anan and Genizi found that pregnancy also had a negative effect on milk yields from the first month post-conception[93]. At the third month of pregnancy, there was a decrease in milk yield by 1.15 kg and by the seventh month there was a decrease in milk yield by 3.73 kg.

2.5.2 Interaction of hormones during pregnancy and lactation

Estrogen and progesterone are produced continuously during pregnancy. Estrogen during pregnancy contributes to the development of the milk-secreting ducts of the breasts. Progesterone is needed for maintaining the endometrium and helps suppress uterine contractions. Prolactin, made in the placenta, is necessary for the secretion of milk by the cells of the alveoli. The levels of prolactin in the blood increases markedly during pregnancy, and stimulates the growth and development of the mammary tissue, in preparation for the production of milk[94]. However, milk is not secreted before parturition because the pregnancy hormones, progesterone and estrogen, block the action of prolactin. After delivery, levels of progesterone and estrogen fall quickly, prolactin is no longer blocked, and milk secretion begins[95]. When a baby suckles, the level of prolactin in the blood increases and stimulates production of milk by the alveoli cells. Oxytocin is the hormone necessary for milk ejection from the breast (let-down reflex) by making the myoepithelial cells around the alveoli contract[96]. This makes the milk, which has collected in the alveoli, flow and then fill the ducts. Oxytocin is produced more quickly than prolactin. It makes the milk that is already in the breast flow for the current feed. Therefore, during pregnancy, milk production is decreased because of the inhibitory effect of high amounts of circulatory estrogen on the release of prolactin[97].

2.6 Conclusion

It is well known that malnutrition is widespread in developing countries and that many deaths under the age of 5 years are related to inadequate dietary intakes. Many determinants of breast milk and complementary food intakes are well researched but there remains a dearth in knowledge about the association between a pregnancy/lactation overlap and a child's dietary intake. This research project aims to help fill this knowledge gap and add insightful information that will inform health professionals about useful recommendations for optimal child feeding.

3 METHODS

3.1 Study site

Peru is the third largest country in South America with a population of close to 30 million[98]. Seventy-six percent of the total population live in urban areas and more than two-thirds of the people living in urban areas currently live in shantytowns; this number is growing at an annual rate of 3%[11]. According to the United Nations Human Settlements Program Report, the situation in Peru is predicted to further urbanize with more people moving to urban areas and living in shantytowns; the estimated rate of change of urbanization from 2010 to 2020 being 1.46%[99].

Peru is divided into 25 regions with geographical variation from the arid plains of the Pacific coast to the peaks of the Andes Mountains to the rainforests of the Amazon Basin. This study was conducted in the capital city, Lima, situated on the desert coast of the Pacific Ocean. Lima has a population of 8 445 200 accounting for approximately 30% of the total population of Peru, with the majority of growth in the city occurring in the periphery of the city centre[11].

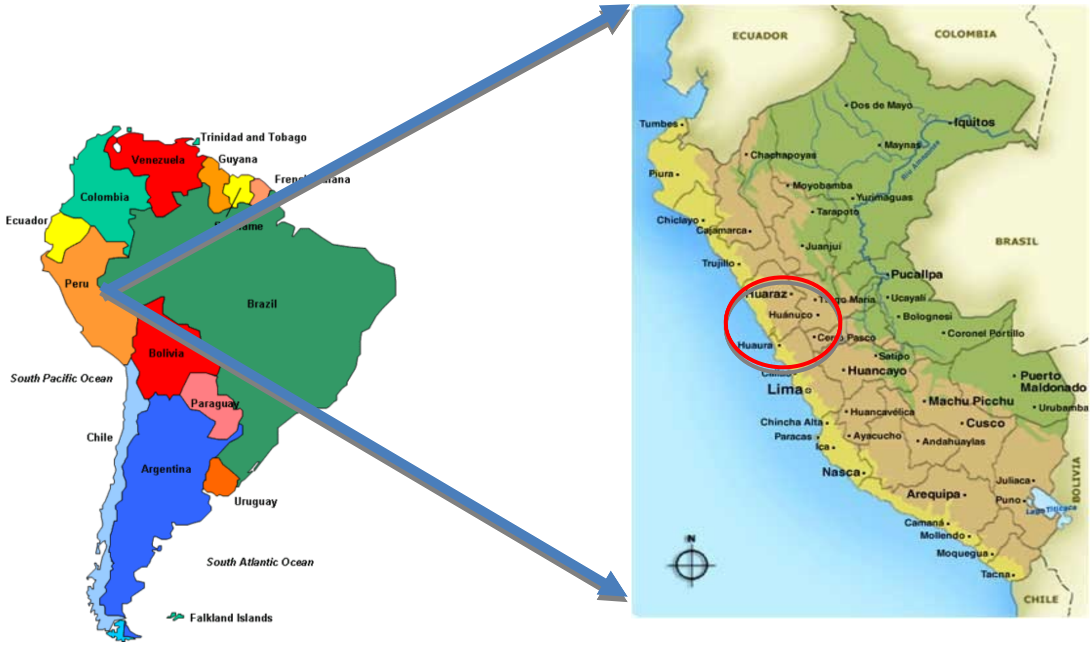


Figure 3.1. Map of Peru with its geographic regions and its location in South America.

Lima is divided into 43 districts, with this study being conducted in the district of San Juan de Lurigancho (SJL) situated in the eastern area of Lima, with a population fast approaching one million inhabitants[100]. The study sites were the peri-urban shantytowns Huascar, Bayovar, and Jose Carlos Mareategui, which are in the eastern-north part of SJL, an area called Canto Grande. It is hard to provide detailed numbers of the exact populations of these communities since they are constantly changing and growing but, based on information from the local hospitals, Huascar is estimated to have a population of about 86 000 people, Bayovar a population of approximately 38 000 people, and Jose Carlos Mariategui a population of approximately 50 000 people.

Canto Grande has been populated for the last 30 years primarily by migrants from the highlands of Peru; most people living in the study area are first- or second-generation migrants who have settled in this area as squatters on the piece of land where they currently live. Although censuses and health surveys have shown that some people in the study area are gradually improving their standard of living, some families live in poor-quality housing with an intermittent water supply and no connected sewage disposal system. Increasing numbers of adults have completed at least primary education, but unstable employment and low incomes are the norm[11].

The study was conducted in coordination with the Instituto de Investigación Nutricional (IIN), a Peruvian non-governmental organization, which has had a permanent research presence in this community since 1980 making the setting favourable for conducting this study. A field center was located in the center of Huascar where various other projects were being conducted by the IIN.

3.2 **Study design**

This was a cross-sectional study examining the association of an overlap of breastfeeding and early pregnancy on the dietary intake of infants and young children. Two groups of mother-child pairs, one where the mother was currently both breastfeeding and pregnant, and one where the mother was breastfeeding but not pregnant, were compared in terms of the difference in dietary intake of the infant/toddler.

3.3 **Participants and selection criteria**

The IIN continually updates a house-to-house census of all blocks in the shantytowns of Huascar, Bayovar, and Jose Carlos Mareategui. This community census list provides information such as the presence of women living with a child less than 36 months old, and pregnancy information including expected due date, age, name, and contact information. This community census list, in addition to self-referrals and mothers identified on the street, provided a list from which mother-child pairs were chosen for the study.

3.3.1 Sample Size

Sample size calculations were made using the following formula[101]:

$$N= [(1/q_1 + 1/q_2) S^2 (z_\alpha + z_\beta)^2] / E^2$$

Where N= the total number of participants in each group

q_1 = the proportion of participants in group 1

q_2 = the proportion of participants in group 2

S = the standard deviation of the outcome variable

z_α = the standard normal deviate for α

z_β = the standard normal deviate for ß

E = the expected effect size of the outcome variable

Assumptions: 2-sided test, equal proportions of participants, z_α=1.96, z_β=0.84
Desired precision: two-sided alpha (α) of 0.05
Desired power: 0.80, therefore a beta (β) of 0.20

Objective: To determine the association of concurrent lactation and pregnancy with the dietary intakes of Peruvian children under the age of 36 months.

Outcome variable: Difference in dietary intake expressed as total energy intake by child weight (kcal/kg) between groups.

N= 157 or 79 in each group (breastfed child with a pregnant mother and breastfed child with a non-pregnant mother).

In Canto Grande the annual birth cohort has been about 10,000 for many years and other field studies have shown that 40% of all pregnant women have a child less than four years of age, therefore this sample size seemed realistic[9].

Standard Deviation: There were no previous studies that analyzed the association of pregnancy status and child dietary intake. Therefore a search of the literature was performed to locate other studies of comparable design and which analyzed the dietary intake of breastfeeding infants or young children. WHO recommends that healthy, breastfed young children 12-23 months have a total dietary intake of 894 kcal/d [27]. Similarly, Fisher *et al.* found that the daily energy intake, based on the mean of three day weighed food records, was 885 ± 197 kcal for 12-23 mo-old toddlers from the United States[102]. Therefore, the *standard deviation used was 197*.

Effect size: A 10% difference in total energy intake was determined to be a clinically important difference. This signified an *effect size of 88* kcal/d. These data were based on a study by Marquis *et al.* which found a significant 8% decline in breast milk intake in 1-mo-old newborns who had a mother who breastfed during her pregnancy compared to 1 mo-old newborns who did not have a mother who breastfed during her pregnancy[9].

Effect size rationale: This 88 kcal/d energy deficit that could occur in the child if the mother continues to breastfeed throughout her pregnancy represents an important reduction in energy intake. If a child has a daily 88 kcal-lower energy intake, this could result in a deficit of 2,728 kcal over one month. If continued over a six-month period, this would result in a lower energy intake of 16,368 kcal. Therefore, over a six-month period of a mother's pregnancy, a breastfed child will gain 2.12 kg less than a breastfed child with a non-pregnant mother. The graph produced by WHO demonstrates that a child of 12 months with a WAZ of zero (reference mean) should weigh 9.2 kg and that a child of 18 months with a WAZ of zero should weigh 10.4 kg[103]. If a child fails to gain the 2.12 kg over the six-month period, the child at 18 months would have a WAZ of -1.9. Additionally, if a child had a WAZ below the reference mean at 12 months, this same child at 18 months would be considered underweight since their WAZ would be below -2.0.

3.3.2 Eligibility criteria

Inclusion criteria for the mothers:

1) Must be at least 18 years old to provide independent consent;
2) Must have at least one child under 36 months of age. In the case where the mother had more than one child under 36 months of age, the youngest was included in the study;
3) Must be breastfeeding their child a minimum of once a day at the time of enrollment;
4) Must be living with her child under 36 months of age;
5) Must be in either her 1st or 2nd trimester of pregnancy or not knowingly pregnant.

Inclusion criteria for the child:

1) Must be under the age of 36 months;
2) Must be breastfeeding;
3) Must not have any congenital malformations or complications that would hinder their ability to breastfeed.

3.3.3 Enrollment

All mother-child pairs who met the inclusion criteria and where the mother was in her first or second trimester of pregnancy, referred to as early pregnancy (EP), were invited for the study. The EP-pairs were visited at their homes to confirm that the child was living there and continued to breastfeed and, if this was confirmed, they were invited to participate in the study. Once these pairs were enrolled in the study, mother-child pairs who met the inclusion criteria and where the mother was not knowingly pregnant (NP) were identified. A NP-pair was matched to an EP-pair based on the child's age (± 2 weeks), to ensure similar age distributions of the two groups. These matched children were randomly selected from the census list, since there were more NP-pairs identified in the census list than EP-pairs.

When the fieldworkers visited the house, they informed the mother about the study's purpose, procedures, potential risks and benefits as well as their rights. They were also informed that their participation was completely voluntary and that no compensation was provided. Written consent was obtained and acknowledged (Appendix 8.1). After the mother agreed to participate in the study and agreed to a date for the study observation, the field workers proceeded with data collection.

3.4 Data collection procedures

Data collection began in September 2010 and continued until December 2010. The field research team responsible for the data collection was made up of four people: the researcher who designed and coordinated the study; two paid fieldworkers, one who carried out the observations and the other who was responsible for enrollment of participants; and one intern, who was responsible for the dietary recalls and, occasionally, observations and enrollment.

3.5 Pretesting of tools and fieldworker training

The researcher and fieldworkers were responsible for carrying out the quantitative data collection. The two fieldworkers were recruited and hired through the assistance of the IIN. The IIN had a list of experienced fieldworkers who had worked for previous research projects and who were familiar with the data collection procedures and with the community. The intern, who acted as a fieldworker, was completing an internship as required for her nutrition degree at a local university. These fieldworkers, who had completed at least a high school education, were trained for this project with the specific data collection methods and on enrollment of participants. All fieldworkers and the researcher spoke Spanish, the local language.

The researcher and fieldworkers were all standardized in data collection techniques prior to commencing the study. The researcher led a two-week intensive training course for the fieldworkers, with help from experienced nutritionists from the IIN who were familiar with all data collection tools. To begin, the study was explained and standard procedures were reviewed. Practice of balance calibration and child weighing occurred. The researcher and one fieldworker were standardized in anthropometric measurements. Ten children from the community were invited to participate in the standardization, five children < 24 months of age and five children of 24-36 months of age. The researcher and fieldworker were standardized with the IIN's nutritionist who specializes in anthropometric measurements.

Further training consisted of the fieldworkers cooking and weighing foods with a trained nutritionist. Several practice meals were created for weighing food practice. Once the fieldworkers were comfortable with all data collection tools, a period of supervised practice observations in the field were conducted until they could perform all tasks independently. They each conducted two different observations and dietary recalls with different age groups (<24 months and 24-36 months) with an experienced fieldworker.

3.6 Data collection

Before data collection commenced, each participant was assigned a numeric code; this code was used on all forms instead of the participant's name. All documents linking the participants' names to their numeric codes were accessible only to the study investigators. All records containing participant information were kept secure; hard-copy documents were kept in a locked file cabinet, and electronic files were password-protected. The researcher obtained all collected data from the field workers at the end of each working day and stored the data in the field center of the IIN.

All of the research took place at the participants' homes where the fieldworkers arrived around 9 am and stayed for six hours. Time of arrival varied depending on the distance from the field center, distance from the end of road access to the house, and accessibility of the house (walkways, stairways, or rocky hillsides).

Data collection in every house was conducted over two days: the first day, a 6-hour observation was conducted and the second day, an 18-hour dietary recall procedure was administered. Together, the 6-hour observation and the 18-hour dietary recall formed a complete 24-hour period of dietary intake.

3.7 Day 1: 6-hour observation

During the home visit, the child's food, liquids, and breast milk were measured. Additionally, socioeconomic and demographic information and anthropometric data were collected. The following sections 3.7.1-3.7.4 describe how these data were collected.

3.7.1 Food and liquid measurement

Intake of food and liquids were measured through standardized test weighing methods[102, 103]. All foods were weighed to a precision of 1g using the Ohaus CS Compact Digital Kitchen Scale. All the scales were validated with standard weights at the beginning of each day. The IIN has a continuously updated Peruvian food composition table, which includes a list of all foods and liquids. When food items were purchased, a list of ingredients was obtained either from the packages (when available), the food vendor, or from the community. Macronutrient estimations of the diet were made using the food composition table.

To quantify the consumption of foods other than breast milk, each food item or prepared mixed-food recipe, was weighed before being offered to the child. In the case of mixed-food recipes, each ingredient was weighed as it was included in the preparation, by first weighing the serving plate, then the first food item and plate, until the final cooked or prepared weight of the recipe was noted. The amount of the prepared recipe given to the child was weighed so that the amount of each individual ingredient could be computed. When recipes and food items were served together on a plate, they were separated and weighed individually. At the end of the feeding event, any unconsumed food was subsequently weighed individually and recorded. The total weight of food consumed by the child was calculated by subtracting the weight of the food after the feeding, from the weight of the food before the feeding. Forms for the intake of food and liquid other than breast milk and for recipes can be found in Appendix 8.2 and Appendix 8.3, respectively.

3.7.2 Breast milk measurements

Breast milk intake was measured by the test weighing method (i.e., weighing the child before and after each feed in the same clothing), using an electronic digital balance and recording to the nearest 1.0 g[104]. Total milk intake was corrected for a 3% insensible water loss[105, 106]. Information such as the difference in the weight of the child before and after the feed, the time that the child was nursing at the breast, the number of feeding episodes, location of the breastfeed, and if the child slept during the breastfeed, were also collected. The form for recording the intake of breast milk can be found in Appendix 8.4.

To calculate 24-hour breast milk intakes, unobserved breast milk intake was estimated from the number of feeds recalled multiplied by the average intake per observed feed in the study.

3.7.3 Household socioeconomic and demographic information

Interviewer-administered questionnaires were used to obtain information about the mother, the household, socioeconomic and demographic characteristics, child's birth information, and, in cases where the mother was pregnant, pregnancy information. The mother's age, birthplace, language spoken, marital status, education level, occupation, and health insurance type were recorded. Information related to the house included ownership of residence, number of rooms, water source, sewage, cooking gas, electrical source, and ownership of electrical material possessions that work, such as an oven or a television. Additionally, household information including who was the primary provider and his/her occupation, household size, number of people who shared food on a regular basis, number of people who contributed financially for food, money spent daily on food, purchasing food from community kitchens or restaurants, and participation in the Glass of Milk program and/or other Non-Government Organizations or church/religious programs were collected. Information about the child included birth date, sex, birth weight, birth length, and if premature (based on mother's recall). Frequency of breastfeeding during the night and day was recorded. Pregnancy information included the most recent ultrasound information and the date of the mother's last menstrual period based on mother's recall. The questionnaire and respective codes can be found in Appendix 8.5 and Appendix 8.6, respectively.

3.7.4 Anthropometric data

The child's age was documented using either a birth certificate or a health card whenever possible. For height and weight measurements, two measurements were taken in immediate succession and the average of the two measurements was used for the analysis. Standard anthropometric measurement techniques were used[107]. Child weight measurements were taken in the morning of the observation before the child was served his/her lunch. Weight measurements were conducted by weighing the child in light clothing using an electronic digital balance and recording to the nearest 1 g. For children under 24 months, the child's length was taken using a stadiometer with the child lying on its back without shoes, socks, or diapers. This stadiometer was locally made and consisted of a baseboard and an adjustable headboard. For children 24-36 months, height was measured using a stadiometer with the child standing upright and barefoot. The stadiometer was mounted at a right angle on a level floor and against a straight, vertical surface such as a wall or pillar. Height/length measurements were recorded to the nearest 0.1 cm. The anthropometric measurement form can be found in Appendix 8.2.7.

3.8 Day 2: 18-hour dietary recall

During the dietary recall in the child's home, the child's food, liquids and breast milk intake were recorded. The USDA 5-step multiple-pass approach was used for the interviewing technique[108]. The steps of this 24-hour dietary recall were as followed:

1) Quick list- used to collect a list of foods and drinks consumed in the previous 24-hours;
2)Forgotten Foods- probe for foods forgotten during the quick list;
3)Time and Occasion- collect time and eating occasion for each food;
4)Detail Cycle- for each food, collect detailed description, amount, and additions, and follow it by reviewing the 24-hour day and;
5)Final Probe- final probe for anything else that could have been consumed.

In cases where the mother still had some of the recipe or food available, recipes of the foods were created and recorded and the portions for the child were weighed according to what the mother remembered giving to the child. The mother was asked to also show how much food the child did not finish. In cases where food was not available, recipes or food of similar weight or water were used.

The same forms used for the food and liquid consumption and the recipes were used to document the dietary recall. Breastfeeding was documented on the consumption of foods and liquids form during the dietary recall to provide information on feeding frequency.

3.9 Statistical analysis

3.9.1 Data entry

Data were entered and cleaned using Microsoft FoxPro 6.0.

3.9.2 Dependent variables

The primary dependent variable examined was the child's total energy intake. Secondary dependent variables included 1) breast milk intake, 2) complementary food intake and 3) anthropometric indicators.

Child total energy intake: Complementary foods in grams were converted into energy (kcal) using the food composition table created by the IIN[109]. Breast milk intake in grams was extrapolated from the 6-hour observation and then converted into energy[104]. Energy values from breast milk and complementary foods were added together to create a new variable that encompassed total energy intake. Since children's weight affects their energy intake, another variable, the child's energy intake per kilogram of weight of the child, was created. PROC UNIVARIATE and stem and leaf plots were used to detect outliers and to check for normality. All outlying points were examined to determine whether errors had been made in data entry. Since no errors were found, all outlying points were included in the analyses. A Shapiro-Wilk test revealed that energy intake by child body weight was not normally distributed, therefore, non-parametric tests were conducted during bivariate analysis. Once the appropriate total energy intake model (multiple linear regression) was fitted, the residuals for this model were tested for normality and were found to be normally distributed.

Complementary food intake: Complementary foods in grams were converted into energy (kcal) using the food composition table created by the IIN[109]. PROC UNIVARIATE and stem and leaf plots were used to detect outliers and to check for normality. A similar analysis as above for total energy intake was performed and once the complementary food intake model was fitted, the residuals for this model were tested for normality and were found to be normally distributed.

Breast milk intake: Breast milk intake in grams was converted into kilocalories based on conversion information from the book *Handbook of Milk Composition*[104]. PROC UNIVARIATE and stem and leaf plots were used to detect outliers and to check for normality. A similar analysis as above for total energy intake was performed and once the breast milk intake model was fitted, the residuals for this model were tested for normality and were found to be normally distributed.

Anthropometric Measurements: Child length/height and weight data were computed into weight-for-age (WAZ), weight-for-height/length (WHZ) and height/length-for-age (HAZ) z-scores using World Health Organization Anthro, version 3.2 2010 (WHO, Geneva, 2010). The Z scores were first analyzed as continuous data and then transformed into categorical data. Children were classified as underweight if their WAZ was less than -2 standard deviations, classified as wasted if their WHZ was less than -2 standard deviations, classified as stunted if their HAZ was less than -2 standard deviations, and classified as overweight if their WAZ was more than +2 standard deviations. Descriptive analysis revealed that HAZ, WAZ, and WHZ were normally distributed, therefore, parametric tests were conducted during the analysis. Outlying points were detected using stem and leaf plots and were examined to check for potential errors. Since no data entry errors were discovered the outliers were included in the analyses.

3.9.3 Independent variables

The mother's pregnancy status was defined by gestational age. For women who had had an ultrasound, the information provided about the gestational age at the time of the ultrasound was used to calculate their gestational age at the time of the observation. This was done by calculating the difference in days between the ultrasound and observation and adding it to the provided gestational age from the ultrasound. In cases where the mother did not have an ultrasound, the method of estimating gestational age was by calculating the time between the first day of the last menstrual period and the date of the observation[110, 111].

Other independent variables considered in the statistical analysis were based on the *a priori* conceptual framework and published literature. These included socioeconomic and demographic factors including household information (family size, daily food expenditure, number of rooms, material of walls and floors, water, electricity, number of material possessions), maternal characteristics (age, occupation, education, civil status, birth place), child information (age, sex, weight, height, premature) and feeding characteristics (feeding frequency, energy density, time spend at the breast). Feeding frequency was defined as the number of feeds in the 24-hour period. A feed was defined as any time the child began consuming foods, liquids, or breast milk and then, according to the fieldworker, the child ended the consumption of the same item. Energy density was calculated by dividing the energy of foods and liquids by the grams of food and liquids. When appropriate, variables were collapsed or grouped together. A composite variable was created to demonstrate household material assets. Each asset had a value of one and the total number of assets were added together for a score out of nine [112]; the nine material assets were: television, radio, blender, stereo system, computer, sewing machine, washing machine, microwave, and refrigerator.

3.9.4 Univariate, bivariate, and regression analysis

SAS software (version 9.2; SAS Inc, Cary, NC) was used for data analysis. Descriptive analyses were used to determine means, ranges and measures of dispersion for all measured variables. Means and standard deviations were generated for continuous variables and frequency distributions were generated for categorical variables. One-way analysis of variance (ANOVA) was performed for continuous variables that were normally distributed. For continuous variables that were not normally distributed Mann-Whitney non-parametric tests were performed to compare the distribution of ranks and to detect differences in median values between groups. Categorical data were analyzed using chi- square tests; however, when tables had less than five values, Fisher's exact tests were conducted. Bivariate tests between independent variables and outcomes of interest (total energy intake, breast milk intake, complementary food intake, and anthropometric indicators) were performed. Associations that were found to be statistically significant or approached significance ($p < 0.25$) were tested in the models. For all tests, statistical significance was set at <0.05.

SAS programs PROC GLM and PROC CORR were used to detect any correlations between variables. One technique used to detect multicollinearity among the independent variables was by examining the tolerance collinearity diagnostic statistics. This study used a significance of <0.4 to suggest multicollinearity.

PROC GLM and PROC REG were used for multiple linear regression to determine the association between the primary dependent variable, total dietary intake, and important independent variable from the *a priori* conceptual framework and published literature. Additionally, multiple linear regression was used to determine associations between secondary dependent variables: 1) breast milk, 2) complementary food intake, 3) total energy intake, and 4) HAZ, and important independent variables.

The independent variables included the main variable of interest, pregnancy status, in addition to variables from socioeconomic status (material of walls and floors, number of material assets) household characteristics (family size, number of household members per room, food expenditure), mother characteristics (age, occupation, education), child characteristics (sex, weight, height), and feeding characteristics (feeding frequency, energy density, time spend at the breast). To construct multivariable models, variables that had a significance under 0.1 or that were in the *a priori* conceptual framework were considered. Any variable that changed the beta coefficient by more than 10% was kept in the model. For all tests, statistical significance was set at <0.05.

PROC GLM provided regression estimates of continuous variables and least square means for categorical variables. The importance of each variable was examined in two ways: a) by computing a partial R-squared, by dividing the Sums of Squares for the given effect (SS) by the Total Corrected Sums of Squares (TCSS); and b) by computing standardized coefficients, calculated by dividing the dependent variable and each independent variable by their respective standard deviations, which standardizes all variables to have a standard deviation of unity.

3.10 **Ethics approvals**

Ethics approvals were obtained prior to commencing fieldwork by the Research Ethics Board-III of McGill University (Appendix 8.8) and by the institutional review board of the IIN (Appendix 8.9).

3.11 **Timeline of research**

Table 3.1: timeline of research activities

	Year/ Month											
	2010								2011			
Activities	M	J	J	A	S	O	N	D	J	F	M	A
Develop draft data collection tools	X	X										
Ethics reviews and approvals	X	X	X									
Travel to Peru					X							
Field test data collection tools					X							
Data collection					X	X	X	X				
Data entry and data cleaning					X	X	X	X				
Return to McGill								X				
Data analysis									X	X	X	X

3.12 **Additional late pregnancy group for analysis**

To examine the relationship between pregnancy status and child dietary intake further, and given the availability of another data set, which included breastfeeding during their late, third trimester of pregnancy (LP), an additional comparative analysis was performed. This analysis included data from the NP group and EP group, as described above, and additionally, the LP group, thus, three groups of breastfeeding children. The data from the LP group were collected as a part of a larger study by Marquis and colleagues (unpublished data). This larger study was a prospective cohort study of pregnant women, their young children, and their newborn babies. The objective of this study was to determine the effect of a lactation-pregnancy overlap on infants through six months; this was accomplished by collecting data at three time periods: pregnancy (from 32 weeks), birth, and through their first six months of life. The information collected during pregnancy included sociodemographic and economic information, breastfeeding practices, maternal anthropometry, maternal morbidity, toddler morbidy and anthropometry, and toddler weighed dietary intake. By including this extra group of mother-child pairs, an analysis that could examine the associations of all phases of pregnancy status and child dietary intake was possible.

3.12.1 Participants

The LP mother-child pairs were selected and identified from the IIN community census of Huascar, Bayovar, and Jose Carlos Mareategui. The eligibity criteria for the LP mother-child pairs were:

1) Must be at least 18 years old to provide independent consent;
2) Must have at least one child under 36 months of age. In the case where the mother had more than one child under 36 months of age, the youngest was included in the study;
3) Must be breastfeeding their child a minimum of once a day at the time of enrollment;
4) Must be living with her child under 36 months of age;
5) Must be in her 3rd trimester of pregnancy.

All LP mother-child pairs who met the inclusion criteria were invited for the study. The LP mother-child pairs were visited at their homes to confirm that the child was living there and continued to breastfeed and, if this was confirmed, they were invited to participate in the study.

3.12.2 Data collection

The fieldworkers were recruited and hired with the assistance of the IIN and were trained by nutritionists from the IIN and the study coordinator. The LP data used in this analysis was collected from January 2009 until December 2010.

All data collection tools and forms used have been explained previously in section 3.6: Data collection. All of the research took place at the participants' homes where the fieldworker conducted a 24-hour observation instead of the 6-hour observation as explained in section 3.6. During the 24-hour observation, the fieldworker measured all breast milk and complementary foods consumed in addition to anthropometric data, and household socioeconomic and demographic data.

3.12.3 Statistical analysis

For the additional comparative analysis with all three groups, data from the 6-hour observation period for the NP and EP groups were used and a time period from 9:00 AM-3:00 PM was selected from the 24-hour observation of the LP group. All breast milk and complementary foods consumed in this 6-hour observational time period were used in the analysis.

All data were entered, cleaned and analyzed with the same programs as described in section 3.9: Statistical analysis. The one exception is that with this additional comparative analysis, there are three groups instead of two groups; therefore a Kruskal-Wallis test was conducted for non-parametric data in univariate and bivariate analyses. PROC GLM was used for multiple linear analysis where the NP group was considered the reference group. When using PROC REG for standardized coefficients, dummy variables were created for pregnancy status, again with the NP group being the reference group.

4 RESULTS

4.1 Study participants

This first analysis is only between the EP and NP groups. Between July 2010 and December 2010, a total of 3320 breastfeeding children under 36 months were identified in the community census (Figure 4.1), of whom 49 had mothers who were in their 1st or 2nd trimester of pregnancy. Nine mothers no longer met the inclusion criteria at the time of enrollment, therefore 40 breastfeeding children with a mother in her 1st or 2nd trimester of pregnancy were invited to participate in the study. Thirteen refused for reasons including 1) the mother worked/was unavailable and did not want her child to participate in the study without herself being present and 2) the mother, or a household member, did not feel comfortable with a fieldworker in their home for reasons such as overcrowded houses and invasion of personal space.

Of the 3320 breastfeeding children, 3271 had a mother who was not knowingly pregnant. Children from the NP mothers were matched for age with children from the EP mothers. Sixty-eight NP mother-child pairs were randomly selected, however 12 pairs no longer met the inclusion criteria at the time of enrollment and therefore, 56 pairs were invited to participate in the study. Twenty-nine NP mother-child pairs refused for similar reasons as the EP mother-child pairs. A total of 54 children were included in the analysis: 27 children with a mother in her 1st or 2nd trimester of pregnancy and 27 children with a non-pregnant mother.

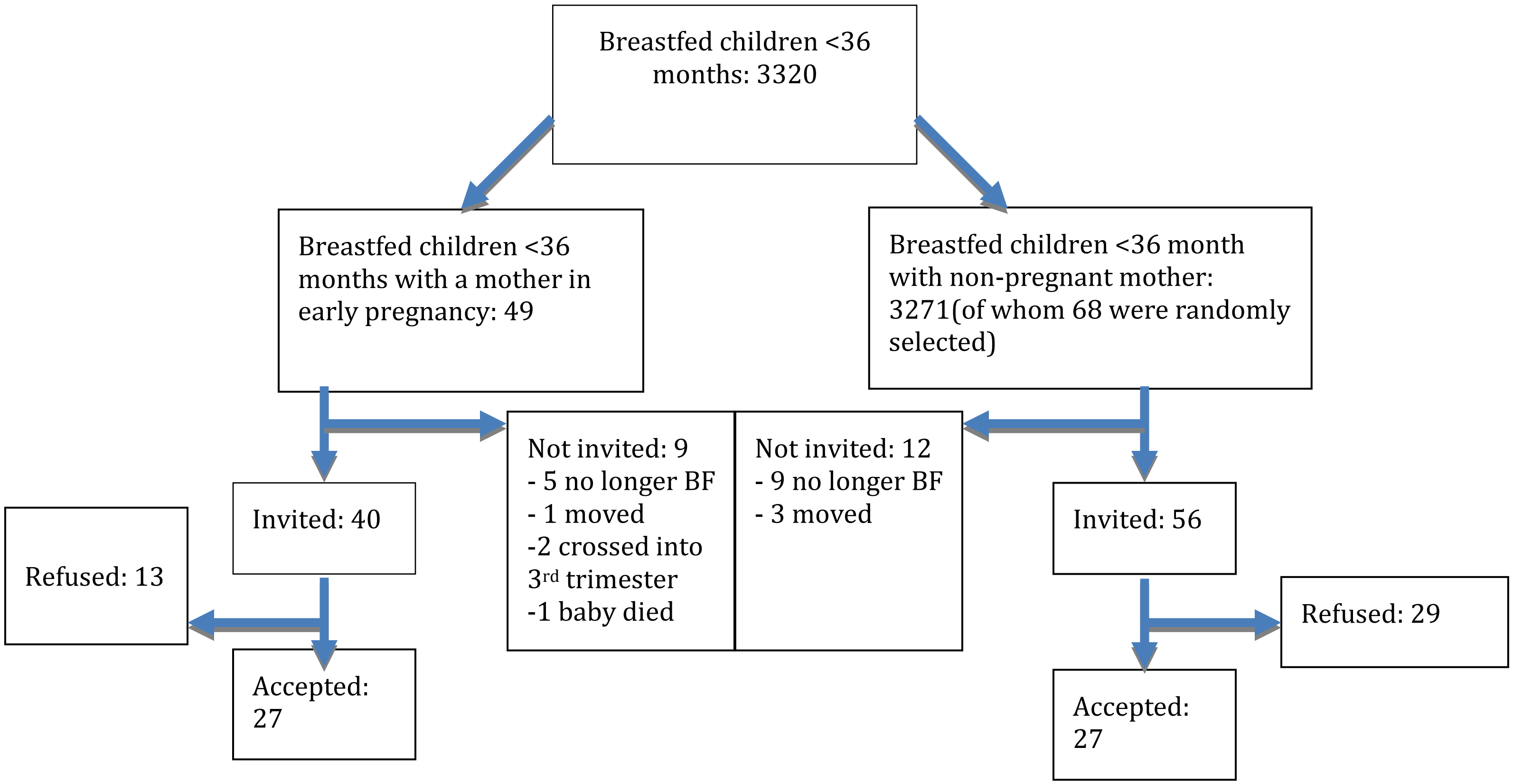


Figure 4.1. Flow chart of non-pregnant and early pregnancy mothers' enrollment

4.2 Maternal characteristics

Analysis by pregnancy status (EP vs NP groups) did not reveal important differences in maternal characteristics (Table 4.1). The median age of all mothers in this study was 25 years with the youngster mother being 18 years old and the oldest being 44 years old. Almost two-thirds of the mothers were born on the coast of Peru (63%, n=34), while 30% (n=16) of mothers were born in the highland region and only 7% (4) were born in the jungle region. Spanish was the most common first language spoken but a small percentage spoke Quechua or another indigenous language as their first language. Over half the mothers had completed high school and 11% of these mothers continued to study at a higher level. Among the mothers who did not complete high school, 26% (n=14) attended high school but did not complete it, 9% (n=5) completed primary school but did not attend high school, and 8% (n=4) attended primary school but did not complete it. The majority of mothers were married or lived with their partner and 85% (n=46) of the mothers identified their husband/partner to be the primary provider for the family. Husbands/partners of the mothers typically engaged in informal work such as drivers of taxis or public buses (22%, n=12), vendors on the street or in informal markets (17%, n=9), construction (17%, n=9), and labourers (13%, n=7). Only two households were female-headed and 6 households considered the maternal/paternal grandparents of the child to be the head of the household. Less than half of the mothers had health insurance and if they did have insurance, 65% (n=15) had Integral Health Insurance (SIS) which is a targeted, fully subsidized health insurance for the poor and 30% (n=7) had EsSalud whose beneficiaries are mainly formal sector workers and their families. Just under half the mothers were employed with the most common type of employment being as a vendor on the street or in informal markets.

The pregnant mothers were on average in their 20th week of pregnancy, with the earliest pregnancy being 9 weeks and the latest pregnancy being 27 weeks. There were seven mothers in their first trimester of pregnancy and twenty mothers in their second trimester of pregnancy.

Table 4.1: Characteristics of 54 mothers of children <36 months of age in low-income peri-urban communities of Lima, Peru, by category of pregnancy status[1]

Maternal characteristics	All (n=54)			Pregnancy Status[3]						p-value[2]
				EP group (n=27)			NP group (n= 27)			
Age (y)	26.7	±	6.7	26.0	±	5.8	27.4	±	7.5	0.43
Born on the coast	63.0		(34)	63.0		(17)	63.0		(17)	1.00
Lived in Lima (y)	19.9	±	9.9	18.6	±	9.3	21.2	±	10.5	0.34
Lived in study site (y)	16.9	±	10.0	16.6	±	10.0	17.3	±	10.0	0.78
High school complete or more	57.4		(31)	59.3		(16)	55.6		(15)	0.96
Married/common law	90.7		(49)	96.3		(26)	85.2		(23)	0.16
Currently working[5]	18.5		(10)	18.5		(5)	18.5		(5)	1.00
Worked in the last year	37.0		(20)	48.1		(13)	25.9		(7)	0.09
Had health insurance	42.6		(23)	40.7		(11)	44.4		(12)	0.78

[1]Data are presented as mean ± SD or % (N)
[2]Data for this table were tested for significant differences using one-way ANOVA, Mann-Whitney non-parametric test, chi-square and Fisher's Exact Test, when appropriate
[3] EP= early, 1st and 2nd trimester, pregnancy; NP= non-pregnant

4.3 Household characteristics

In the study sites the majority of households had electricity in their homes, while small percentages used their neighbour's electricity (11%, n=6) or candles (7%, n=4). Cement floors were the most common compared to having an earth floor (28%, n=15). Walls were generally permanent, made from brick (finished with cement fill or unfinished without the cement fill) as opposed to plywood

(11%, n=6) or straw mat (24%, n=13). The majority of the households had potable water inside their house while 17 % (n=9) used a public tank and 11% (n=6) used their parents or neighbours source of water. Having a toilet with connected drainage was the most common form of a bathroom, while the second most common form was having an outhouse without drainage (22%, n=12). The only significant difference between groups was wall material, with more EP households having a permanent wall than NP households (Table 4.2).

Over half of the households lived with extended family with the average household being made up of five people. The average number of household members per room (excluding the kitchen and bathroom) was just over three persons. Most households contained 2-3 assets that worked; the most common asset was a television and then a blender. Average daily household food expenditure was approximately 19.11 PEN (2.77 PEN= $ 1.00 US). On average, more people in the NP group (1.8 ± 1.0) contributed financially to food expenditure than the EP group (1.3 ± 0.8), which was approaching significance ($p = 0.06$). Approximately five people shared meals together on a daily basis; the NP group had one more adult-shared meals with the household than the EP group ($p = 0.01$). Over one third of the households participated in the government-funded 'Glass of Milk' program that provides milk to children and pregnant women; both groups had similar numbers involved with the program.

Further analysis by pregnancy status revealed important differences in household socioeconomic variables. In general, households of the EP group tended to have worse living conditions than the households of the NP group. The EP group was four times less likely to have more than four electrical material assets compared to the NP group. On average, the EP group had one less room in their house than the NP group.

Table 4.2: Characteristics of 54 households of children <36 months of age in low-income peri-urban communities of Lima, Peru, by pregnancy status[1]

Household characteristics and indicators of wealth	All (n=54)			Pregnancy Status[3]						p-value[2]
				EP group (n=27)			NP group (n= 27)			
Electricity	81.5		(44)	74.1		(20)	88.9		(24)	0.16
Sewage	64.8		(35)	55.6		(15)	74.1		(20)	0.15
Finished floor	72.2		(39)	70.4		(19)	74.1		(20)	0.76
Permanent walls	64.8		(35)	51.9		(14)	77.8		(21)	0.04
Gas cooker	98.1		(53)	96.3		(26)	100		(27)	0.30
In-house water	72.2		(39)	70.4		(19)	74.1		(20)	0.76
Household assets:										
Refrigerator	40.7		(22)	29.6		(8)	51.9		(14)	0.10
Television	90.7		(49)	85.2		(23)	96.3		(26)	0.15
Radio	37.0		(20)	25.9		(7)	48.2		(13)	0.09
Blender	48.2		(26)	40.7		(11)	55.6		(15)	0.28
Sound system	40.7		(22)	29.6		(8)	51.9		(14)	0.06
Washing machine	16.7		(9)	14.8		(4)	18.5		(5)	0.27
Sewing Machine	7.4		(4)	3.7		(1)	11.1		(3)	0.61
Microwaves	13.0		(7)	11.1		(3)	14.8		(4)	0.67
Computer	7.4		(4)	3.7		(1)	11.1		(3)	0.61
Total household assets[4]	3.0	±	1.9	2.4	±	1.6	3.6	±	2.1	0.003
Households with:										
0-1 Assets	27.8		(15)	33.3		(9)	22.2		(6)	
2-3 Assets	42.6		(23)	51.9		(14)	33.3		(9)	0.003
≥4 Assets	29.6		(16)	14.8		(4)	44.4		(12)	

Ownership of residence:										
Owned	29.6		(16)	40.7		(11)	18.5		(5)	
Rented	11.1		(6)	14.8		(4)	7.4		(2)	0.08
Parents owned	59.3		(32)	44.4		(12)	74.1		(20)	
Rooms in home	2.5	±	1.5	2.0	±	1.7	3.0	±	1.3	0.02
Rooms for sleeping	1.8	±	1.0	1.6	±	1.0	2.1	±	1.1	0.05
Members of household	5.2	±	2.3	4.9	±	2.2	5.6	±	2.4	0.24
Members per room[5]	3.3	±	1.5	3.7	±	1.7	3.0	±	1.3	0.12
Daily food expenditure per member (PEN)[6]	4.1	±	1.7	4.3	±	1.7	4.0	±	1.6	0.58
Purchase from community kitchen	44.4		(24)	37.0		(10)	51.9		(14)	0.41
Participate in Glass of Milk program[7]	38.9		(21)	37.0		(10)	40.7		(11)	0.78

[1]Data are presented as mean ± SD or % (N)

[2]Data for this table were tested for significant differences using one-way ANOVA, Mann-Whitney non-parametric test, chi-square and Fisher's Exact Test, when appropriate

[3]EP= early, 1st and 2nd trimester, pregnancy; NP= non-pregnant

[4]Assets score: sum of refrigerator, television, radio, blender, sound system, microwave, computer, sewing machine, and washing machine

[5]Total # of household members divided by total # of rooms in the home

[6]Expressed in New Peruvian Soles (PEN) ($1US= 2.77 PEN)

[7] 'Glass of Milk' is a government-funded program that provides milk to children and pregnant women

4.4 **Child characteristics**

Child characteristics did not differ significantly between groups (Table 4.3). The average age of the children in both the EP group and NP group was 21 months. There were equal numbers of boys (n=15) and girls (n=12) in each group and then mean weight and length/height was not significantly different between the EP group and the NP group. WAZ, HAZ and WHZ did not differ significantly between the EP and NP groups, however, HAZ was approaching significance ($p=0.09$). Only one child was underweight and no child was wasted. There were an equal number of stunted children in the EP group and NP group but twice the number of children in the EP group had an HAZ <-1 SD compared to the NP group, although this was still not significant ($p=0.10$).

Table 4.3: Anthropometry of children <36 months of age living in low-income peri-urban communities of Lima, Peru, by category of pregnancy status[1]

Child Characteristics	**All** (n=54)	**Pregnancy Status**[3]		**p-value**[2]
		EP group (n=27)	**NP group** (n= 27)	
Age (mo)	21.1 ± 5.6	21.0 ± 5.6	21.1 ± 5.7	0.97
Male sex	55.6 (30)	55.6 (15)	55.6 (15)	1.00
Weight (kg)	11.3 ± 1.9	11.0 ± 1.9	11.6 ± 1.9	0.29
Height (cm)	80.7 ± 5.5	80.1 ± 5.3	81.4 ± 5.3	0.40
Weight-for-age Z score	-0.0 ± 1.1	-0.2 ± 1.0	0.2 ± 1.1	0.15
Height/length-for-age Z score	-1.1 ± 1.0	-1.4 ± 1.0	-0.9 ± 0.94	0.09
Weight-for-Height/length Z score	0.7 ± 0.9	0.6 ± 0.9	0.9 ± 1.0	0.28
Stunted (<-2 SD)[4]	18.5 (10)	18.5 (5)	18.5 (5)	1.00

[1]Data are presented as mean ± SD or % (N)

[2]Data for this table were tested for significant differences using one-way ANOVA, Mann-Whitney non-parametric test, chi-square and Fisher's Exact Test when appropriate

[3]EP= early, 1st and 2nd trimester, pregnancy; NP= non-pregnant

[4] Stunting, HAZ <-2 SD WHO International Growth Reference[11]

Multiple linear regression revealed that when HAZ was adjusted for household assets and total energy intake, it was not significantly associated with pregnancy status (p=0.32) (Table 4.4). All significant predictors found in univariate and bivariate analysis were put into the model. They were all put into the preliminary regression models, however, only variables that had a significance of < 0.1 were kept in the final model.

Table 4.4: Multiple linear regression coefficients of determinants of height/length-for-age z-score of children <36 months of age living in low-income peri-urban communities of Lima, Peru

Variable	Standardized coefficient[1]	SE	P value
Early pregnancy	-0.11	0.37	0.32
Household assets (#)	0.39	0.03	0.01
Total energy intake (kcal)	0.27	0.003	0.03

[1]Model: n=54, adjusted r^2= 0.24, SE= 0.87, p=0.0009

4.5 Child's dietary intake

All regression models presented in this section account for all significant predictors found in univariate and bivariate analysis. They were all put into the preliminary regression models, however, only variables that had a significance of < 0.1 were kept in the final model. Parameter estimates were examined for changes upon removal of independent variables, including socioeconomic status and child characteristic (age, sex, weight, and height) variables, but no changes were seen, therefore the final models presented here were determined as the best fitted regression models.

4.5.1 Complementary foods

Out of a total of 245 recipes created and reported during the 24-hour periods, 96% of them were prepared inside the house. Less than four percent of the recipes were bought from the community kitchen or were made by a family member. The most common food eaten by the children was rice with 96% of children consuming it at least once during the day and often twice a day. Rice was most commonly prepared with oil and salt. The second most common food consumed was potatoes with 74% of children consuming them at least once a day. Potatoes were most commonly served in soups and stews. Fruit was consumed at least once a day by most of the children (81%, n=44), with the most common fruits consumed being tangerines and bananas. The most common vegetables consumed by the children were carrots, green peas and corn, again, most commonly served in soups and stews.

Almost all children received some energy from animal source food (ASF) (96%, n=52). Forty-six children (85%) consumed between 120-360 kcal from ASF and the average energy intakes from ASF were 254.3 ± 169.4 kcal and 219.0 ± 124.6 kcal for the NP and EP groups, respectively. ASF provided more than half of the children's consumption of fat and protein (58% and 55%, respectively). The children also received part of their daily requirements of iron and zinc from ASF (1.9 ± 4.4 mg and 2.1 ± 1.6 mg, respectively). The most common ASF consumed during the 24-hour periods were powder, evaporated, or condensed milk (96%, n=52) and chicken (65%, n=34), served in a soup, fried, or in a form of stew. Other common ASF were eggs, beef, and fish. There were no significant differences found in the energy or nutrients provided by ASF between the two groups.

The average feeding frequency from complementary food was 14.2 ± 3.7 feeds/d. Feeding frequency was defined as the number of feeds in the 24-hour time period with a feed being anytime the child began consuming foods or liquids, and then, according to the fieldworker, the child ended the consumption of the same item. Additionally, a feed was not defined by time since last feed. The EP group ate on average 2 times more often than the NP group in a day, which approached significance (15.2 ± 4.1 feeds/d and 13.3 ± 3.2 feeds/d, respectively; $p = 0.06$). There was no significant difference in energy density of complementary food between the EP group and the NP group (0.64 ± 0.19 kcal/g and 0.73 ± 0.24 kcal/g, respectively; $p = 0.15$). The average energy intake during the 24-hour periods from complementary food was not significantly different between the two groups (Table 4.5). Total complementary food intake was further analyzed by examining the differences per kilogram of body weight (kcal/kg); there was still no group difference (EP group: 92.5 ± 28.7 kcal/kg, NP group: 92.9 ± 35.5 kcal/kg, $p = 0.28$).

Table 4.5: Twenty-four hour energy and selected nutrient intakes from complementary foods of children <36 months of age in low-income peri-urban communities of Lima, Peru, by pregnancy status[1]

	All (n=54)			**Pregnancy Status**[3]						**p-value**[2]
				EP group (n=27)			**NP group** (n= 27)			
Energy (kcal)	987.7	±	387.2	1025.6	±	368.5	949.8	±	405.0	0.36
Carbohydrates (g)	159.1	±	70.9	168.5	±	66.3	149.8	±	75.3	0.22
Protein (g/kg)	2.9	±	1.4	2.9	±	1.3	2.9	±	1.5	0.59
Fat (g)	24.8	±	12.2	24.1	±	9.7	25.6	±	14.3	0.88
Fiber (g)	3.4	±	2.5	3.4	±	2.1	3.4	±	2.7	0.54
Vitamin A (RE)[4]	941.8	±	1158.3	917.9	±	934.9	965.8	±	1345.1	0.87
Vitamin C (mg)	79.7	±	81.8	94.3	±	100.5	65.1	±	57.3	0.20
Calcium (mg)	488.4	±	577.1	554.0	±	749.7	422.7	±	322.4	0.62
Iron (mg)	10.8	±	16.3	12.6	±	21.9	9.0	±	7.1	0.94
Zinc (mg)	5.5	±	9.3	6.4	±	12.9	4.6	±	2.8	0.74

[1]Data are presented as mean ± SD

[2]Data for this table were tested for significant differences using Mann-Whitney non-parametric test

[3]EP= early, 1st and 2nd trimester, pregnancy; NP= not-pregnant

[4]RE= retinol equivalent

Multiple linear regression revealed that when complementary food intake per kilogram of body weight (kcal/kg) was adjusted for feeding frequency (feeds/d), energy density (kcal/g), and ownership of residence, it tended to be positively associated with pregnancy status (p= 0.06) (Table 4.6). Parameter estimates revealed that complementary food intake (g) per kilogram body weight increased about 10 g/kg when the mother was in her 1st or 2nd trimester of pregnancy compared to when the mother was not pregnant. The total energy intake per kilogram of body weight was also positively associated with energy density (*b*= 89.7, p=0.001) and feeding frequency (*b*= 3.0, p=0.001). Energy intake per kilogram of body weight was weakly negatively associated with the ownership of current residence.

Table 4.6: Multiple linear regression coefficients of determinants of complementary food energy intake by body weight (kcal/kg) of children <36 months of age during one 24-hour period in low-income peri-urban communities of Lima, Peru

Variable	Standardized coefficient[1]	SE	P value
Early pregnancy	0.21	7.01	0.06
Feeding frequency (feeds/d)	0.36	0.89	0.001
Energy density (kcal/g)	0.50	15.3	<0.001
Owned residence	-0.20	7.1	0.07

[1]Model: n=54, adjusted r^2 =0.46, SE= 23.7, $p<0.0001$

4.5.2 Breast milk

One inclusion criterion for this study was that the mother was breastfeeding at the time of enrollment; two mother-child pairs had a mother who discontinued breastfeeding after enrollment but before the observation, therefore, breast milk information was available from only 52 mother-child pairs, 27 NP mother-child pairs and 25 EP mother-child pairs.

During the 6-hour observation period the children breastfed on average 1.5 ± 1.3 times (Table 4.7). The EP group had almost one fewer breastfeeds than the NP group in the 6-hour observation (p=0.03) but in the 24-hour period, the EP group breastfed almost half as often as the NP group ($p<0.001$). The children spent 11.2 ± 9.3 minutes at the breast during the 6-hour observation, with each feed being on average 8.0 ± 4.3 minutes. The association of pregnancy status and the child's 6-hours breast milk intake (g) approached significance ($p=0.051$).

Table 4.7: Breastfeeding practices of 52 women with a child <36 months of age in low-income peri-urban communities of Lima, Peru, by pregnancy status[1]

Breastfeeding Characteristics	All (n=52)	Pregnancy Status[3] EP group (n=25)	Pregnancy Status[3] NP group (n= 27)	p-value[2]
6-hour breastfeeding frequency	1.5 ± 1.3	1.1 ± 1.2	1.9 ± 1.4	0.03
24-hour breastfeeding frequency*	4.6 ± 2.3	3.2 ± 2.4	5.9 ± 2.3	<0.001
6-hour breast milk (g)[4]	53.3 ± 42.3	41.1 ± 40.8	64.6 ± 43.6	0.05
24-hour breast milk (g)[4*]	167.7 ± 81.4	115.2 ± 83.3	220.3 ± 79.4	<0.001
6-hour time at the breast (min)	11.2 ± 9.3	8.2 ± 9.0	14.0 ± 9.6	0.03

[1]Data are presented as mean ± SD

[2]Data for this table were tested for significant differences using one-way ANOVA or Mann-Whitney non-parametric test, when appropriate

[3]EP= early pregnancy; NP= non-pregnant

[4] With 3% insensible water loss

[*] To calculate 24-hour breast milk intakes, unobserved breast milk intake was estimated from the number of feeds recalled multiplied by the average intake per observed feed in the study

Multiple linear regression revealed that when 6-hour breast milk intake was adjusted for maternal age, maternal education, number of household assets, and energy from complementary food, it was significantly associated with pregnancy status (p=0.02) (Table 4.8). Parameter estimates revealed that breast milk intake decreased about 28 grams in 6-hours when the mother was in her 1st or 2nd trimester of pregnancy compared to when the mother was not pregnant. Breast milk intakes were also associated with other factors. Breast milk intake was negatively associated with maternal age and positively associated with maternal education (*b*= 2.2, p= 0.037 and *b*= 3.7, p= 0.05, respectively). Energy from complementary food in the six hour observation period was not significantly associated with breast milk intake but approached significance (p=0.07).

Table 4.8: Multiple linear regression coefficients of determinants of breast milk intake (g) of children <36 months of age during one 6-hour observational period in low-income peri-urban communities of Lima, Peru

Variable	Standardized coefficient[1]	SE	P value
Early pregnancy	-0.334	11.663	0.02
Maternal education (y)	0.268	1.824	0.05
Maternal age (y)	-0.302	0.919	0.04
Six-hour complementary food energy (kcal)	-0.237	0.0144	0.07

[1]Model: n=52, adjusted r^2= 0.20, SE= 38.7, p<0.01

4.5.3 Total 24-hour energy intake

The energy intake from complementary foods and breast milk were combined to obtain the total energy intake (kcal). The average feeding frequency during the 24-hour periods from both complementary food and breastfeeding was 18.6 ± 4.3 feeds/d. The EP group had more complementary food feedings but fewer breast milk feedings than the NP group; combined the EP group had one fewer feeding than the NP group (18.1 ± 4.6 and 19.1 ± 4.0 feeds/d, respectively; p= 0.34).

Descriptive analysis demonstrated that the average total daily energy intake was 1102.7± 371.7 kcal and 1097.5 ± 395.1 kcal for the EP and NP group, respectively (p= 0.85). Total energy intake was further analyzed by examining the differences per kilogram of body weight (kcal/kg); there was still no significant group differences (EP group: 99.9± 29.5 kcal/kg vs. NP group: 95.9 ± 34.7 kcal/kg, p= 0.37).

Multiple linear regression revealed that when total energy intake per kilogram body weight was adjusted for feeding frequency (feeds/d), energy density (kcal/g), and ownership of residence, it was positively associated with pregnancy status (p= 0.02) (Table 4.9). Parameter estimates revealed that 24-hour energy intake per kilogram body weight increased about 16 grams when the mother was in her 1st or 2nd trimester of pregnancy compared to when the mother was not pregnant. The total energy intake per kilogram of body weight was also positively associated with energy density and feeding frequency (*b*= 91.1, p<0.001 and *b*= 2.3 p=0.004, respectively). Energy intake per kilogram of body weight tended to be weakly negatively associated with the ownership of current residence (*b*= 12.0, p= 0.09).

Table 4.9: Multiple linear regression coefficients of determinants of total energy intake by child body weight (kcal/kg) of children <36 months of age during one 24-hour period in low-income peri-urban communities of Lima, Peru

Variable	Standardized coefficient[1]	SE	P value
Early pregnancy	0.25	6.73	0.02
Feeding frequency (feeds/d)	0.30	0.75	0.004
Energy density (kcal/g)	0.56	17.2	<0.001
Owned residence	-0.19	7.0	0.09

[1]Model: n=54, adjusted r^2 =0.24, SE= 29.05, p= 0.018

4.6 Additional analysis with the late pregnancy group

Given the availability of the LP group data set an additional comparative analysis was performed. This analysis was conducted to see the dietary differences associated with early pregnancy would be more accentuated in late pregnancy.

4.6.1 Study participants

Between February 2009 and December 2010 a total of 79 mother-child pairs were identified who met the inclusion criteria of the study (Figure 4.2). Eleven mothers were not included in the analysis because seven mother-child pairs did not have a 24-hour observation of the breastfeeding child and four mothers delivered their newborn before an observation of the breastfeeding child was scheduled; additionally, 33 mothers refused to participate in the study. Therefore, 35 mothers were enrolled into the study and accepted into the analysis. A total of 89 children between the ages of 10 and 36 months (27 from a NP mother, 27 from an EP mother, and 35 from a LP mother) were included in this analysis.

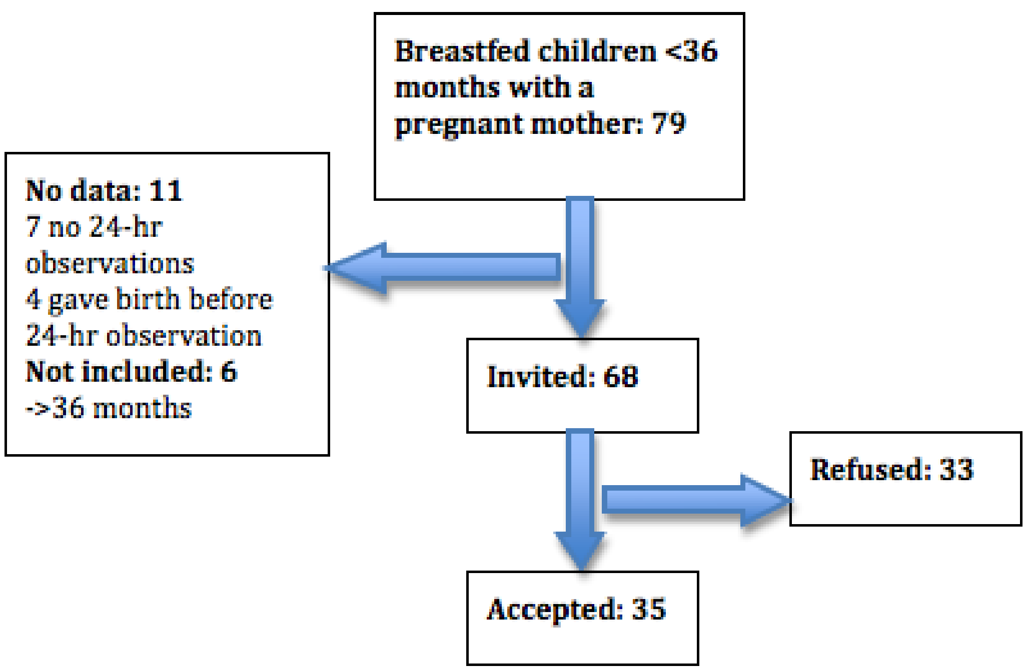


Figure 4.2. Flow chart of late pregnancy mothers' enrollment.

4.6.2 Descriptive differences by pregnancy status

No significance differences were found in maternal characteristics among the LP, EP, and NP groups. There were a few significant differences in household characteristics and child characteristics, which are highlighted in Table 4.10. The differences in a number of household characteristics (i.e., permanent walls, rooms in home, ownership of residence) suggested that the LP group had worse conditions of living than both the EP and NP groups, however, number of household members, daily food expenditure, and ownership of residence were not significantly different. Nutritional status indicators were similar in all groups. There were no significant differences in age, sex, height and weight of the child among the three groups.

All significant predictors found in univariate and bivariate analysis were accounted for and put into the preliminary regression models. However, only variables that had a significance of < 0.1 were kept in the final model. Parameter estimates were examined for changes upon removal of independent variables, including socioeconomic status and child characteristic (age, sex, weight, and height) variables, but no changes were seen, therefore the final models presented here were determined as the best fitted regression models.

Table 4.10: Selected household and child characteristics that differed by pregnancy status of 89 women with a child <36 months of age in low-income peri-urban slums of Lima, Peru[1]

	All (n=89)	**Pregnancy Status[3]** **LP group** (n= 35)	**EP group** (n=27)	**NP group** (n=27)	**p-value[2a]**
Maternal characteristics					
Age (y)	26.6 ± 6.4	26.5 ± 5.9	26.0 ± 5.8	27.4 ± 7.5	0.86
High school complete or more	52.8 (47)	45.7 (16)	59.3 (16)	55.6 (15)	0.85
Household characteristics					
Permanent walls	55.0 (49)	40.0 (14)	51.9 (14)	77.8 (21)	0.01
Gas cooker	82.9 (82)	82.9 (29)	96.3 (26)	100 (27)	0.03
Owned residence	43.8 (39)	48.6 (17)	55.6 (15)	25.9 (7)	0.07
Rooms in home	2.5 ± 1.5	2.4 ± 1.5^{a}	2.0 ± 1.7^{a}	3.0 ± 1.3^{a}	0.01
Household assets:					
Refrigerator	34.8 (31)	25.7 (9)	29.6 (8)	51.9 (14)	0.04
Sound system	31.5 (28)	17.1 (6)	29.6 (8)	51.9 (14)	0.02
Total household assets[4]	2.6 ± 1.7	2.1 ± 1.5^{a}	2.4 ± 1.6^{b}	3.6 ± 2.1^{ab}	0.003
Child Characteristics					
Age (mo)	22.2 ± 6.0	23.9 ± 6.5	21.0 ± 5.6	21.1 ± 5.7	0.10
Male sex	53.9 (48)	51.4 (18)	55.6 (15)	55.6 ± (15)	0.93
Height (cm)	81.4 ± 5.6	82.4 ± 5.6	80.1 ± 5.7	81.4 ± 5.3	0.27
Weight (kg)	11.4 ± 1.9	11.5 ± 2.0	11.0 ± 1.9	11.6 ± 1.9	0.50

[1]Data are presented as mean ± SD or % (N)

[2]Data for this table were tested for significant differences using one-way ANOVA, Kruskal-Wallis non-parametric test, chi-square and Fisher's Exact Test when appropriate

[3]LP= late, 3[rd] trimester, pregnancy; EP= early, 1[st] and 2[nd] trimester, pregnancy; NP= non-pregnant

[4]Assets score: sum of refrigerator, television, radio, blender, sound system, and washing machine

[a]Different superscripts in a row signify a statistical significance <0.05 using the bonferroni test

4.6.3 Complementary foods

Most children received some energy from ASF during the 6-hour observation (91%, n=81); seven children (17%) consumed over 125 kcal from ASF. Out of the 89 children, average energy intakes from ASF were 93.8 ± 74.9 kcal, 84.7 ± 69.0 kcal and 88.1 ± 67.1 kcal for the LP, EP, and NP groups, respectively (p= 0.87). ASF provided about half of the children's consumption of fat and protein (49.7% and 50.9%, respectively). The children also received part of their daily requirements of iron and zinc from ASF (1.0 ± 3.2 and 0.9 ± 0.9, respectively). There were no significant differences found in the energy or nutrients provided by ASF among the groups.

The average feeding frequency from complementary food was 10.7 ± 3.2 feeds, 10.3 ± 3.9 feeds, and 10.6 ± 3.5 feeds in the 6-hour observation, for the LP, EP, and NP groups, respectively (p=0.93). The energy density of complementary food in the 6-hour observation for the LP group was 0.6 ± 0.2 kcal/g, the EP group was 0.6 ± 0.2 kcal/g, and the NP group was 0.7 ± 0.2 kcal/g but this was not significantly different among groups (p= 0.61). There were no significant differences in energy intake or nutrient intakes among the three groups (Table 4.11).

Table 4.11: Six-hour energy and selected nutrient intakes from complementary foods of children < 36 months of age in low-income peri-urban communities of Lima, Peru, by pregnancy status[1]

	All (n=89)	**Pregnancy Status[3]** LP group (n=35)	EP group (n=27)	NP group (n= 27)	p-value[2]
Energy (kcal)	426.7 ± 186.5	429.2 ± 142.7	436.0 ± 182.7	414.5 ± 244.7	0.37
Carbohydrates (g)	73.7 ± 36.3	71.4 ± 23.2	82.2 ± 43.3	68.7 ± 44.2	0.24
Protein (g/kg)	1.2 ± 0.6	1.1 ± 0.4	1.3 ± 0.8	1.2 ± 0.5	0.56
Fat (g)	10.5 ± 6.3	11.4 ± 6.7	10.0 ± 5.9	9.6 ± 5.9	0.40
Fiber (g)	1.8 ± 1.5	1.6 ± 1.3	2.0 ± 1.8	1.8 ± 1.5	0.74
Vitamin A (RE)	436.3 ± 708.6	462.3 ± 857.8	460.6 ± 667.9	372.4 ± 445.6	0.76
Vitamin C (mg)	44.3 ± 61.5	29.2 ± 36.0	64.6 ± 92.2	46.9 ± 53.7	0.13
Calcium (mg)	167.3 ± 401.8	138.0 ± 95.3	266.8 ± 742.9	112.3 ± 73.5	0.63
Iron (mg)	4.9 ± 11.7	3.6 ± 2.0	7.7 ± 21.1	4.1 ± 5.4	0.70
Zinc (mg)	2.6 ± 7.0	2.1 ± 1.5	4.3 ± 13.0	1.7 ± 0.8	0.86

[1]Data are presented as mean ± SD

[2]Data for this table were tested for significant differences using Kruskal-Wallis non-parametric test

[3]LP= late, 3rd trimester, pregnancy; EP= early, 1st and 2nd trimester, pregnancy; NP= non-pregnant

4.6.4 Breast milk

One of the inclusion criterion for this study was that the mother was breastfeeding at the time of enrollment; eight mother-child pairs, six in the LP group and two in the EP group, had a mother who discontinued breastfeeding after enrollment but before the observation, therefore, breast milk information was available from only 81 households.

During the 6-hour observation period the children breastfed on average 1.5 ± 1.4 times (Table 4.12). Descriptive analysis showed a significant difference in breast milk intake among the LP, EP, and NP groups ($p<0.001$) but no significant difference in the time spend at the breast ($p=0.13$).

Table 4.12: Breastfeeding practices of 81 women with a child <36 months of age in low-income peri-urban slums of Lima, Peru, by pregnancy status[1]

Breastfeeding Characteristics	All (n=81)	Pregnancy Status[3]			p-value[2]
		LP group (n=29)	EP group (n=25)	NP group (n= 27)	
6-h breastfeeding frequency	1.5 ± 1.4	1.5 ± 1.6	1.1 ± 1.2	1.9 ± 1.4	0.13
6-h breast milk (g)	33.7 ± 34.7	-1.5 ± 12.8	41.1 ± 40.8	64.6 ± 43.6	<0.001
6-h time at the breast (min)	12.8 ± 14.0	15.6 ± 19.6	8.2 ± 9.0	14.0 ± 9.6	0.13

[1]Data are presented as mean ± SD

[2]Data for this table were tested for significant differences using Kruskal-Wallis non-parametric test

[3]LP= late, 3rd trimester, pregnancy; EP= early, 1st and 2nd trimester, pregnancy; NP= non-pregnant

Further analysis revealed that when breast milk was adjusted for maternal age and maternal education, it was still significantly associated with pregnancy status (Table 4.13). Parameter estimates revealed that milk intake decreased about 27 grams when the mother was in her 1st or 2nd trimester of pregnancy and decreased about 68 grams when the mother was in her 3rd trimester of pregnancy compared to when the mother was not pregnant. Breast milk intakes were also associated with other factors. Breast milk intake was negatively associated with maternal age (*b*= -1.7, p= 0.01) and tended to be weakly positively associated with maternal education (*b*=2.1, p=0.07).

Table 4.13: Multiple linear regression coefficients of determinants of breast milk intake (g) of children <36 months of age during one 6-hour observational period in low-income peri-urban communities of Lima, Peru, by pregnancy status

Variable	Standardized coefficient[1]	SE	P value
Early pregnancy	-0.431	9.11	<0.001
Late pregnancy	-0.730	8.98	<0.001
Maternal education (y)	0.156	1.16	0.07
Maternal age (y)	-0.244	0.62	0.01

[1]Model: n=81, adjusted r^2= 044, SE= 33.2, p<0.0001

4.6.5 Total 6-hour energy intake

Energy intakes from complementary foods and breast milk were combined to create total 6-hour energy intake. The average feeding frequency during the 6-hour observation from both complementary food and breastfeeding was 12.0 ± 4.1 feeds (p=0.57).

Descriptive analysis demonstrated that the average total energy intake for the 6-hour observation was 428.3 ± 142.5, 461.5 ± 184.4, and 457.3 ± 229.1 kcal for the LP, EP, and NP groups, respectively. There was no significant group difference in 6-hour energy intake (p= 0.92). Additionally, there was no significant difference in 6-hour energy intake per body weight among the LP, EP, and NP groups (37.2 ± 11.3 kcal/kg, 41.5± 14.2, 39.9 ± 19.6 kcal/kg; p= 0.56).

Further analysis revealed that when 6-hour energy intake per body weight was adjusted for energy density (kcal/g), feeding frequency (feeds/6-hours), ownership of residence, and numbers of members in the household, it was not significantly associated with late pregnancy status (p= 0.96) and tended to be weakly positively associated with early pregnancy (p=0.08) (Table 4.14). Parameter estimates revealed that energy intake per body weight decreased a non-significant 0.15 kcal/kg when the mother was in her 3rd trimester of pregnancy and increased 5.3 kcal/kg when the mother was in her 1st or 2nd trimester of pregnancy compared to when the mother was not pregnant, although there was a positive trend . Energy intake per body weight was associated with other factors. Energy intake per body weight was negatively associated with ownership of residence (*b*=-6.7, p=0.02) and members in household (*b*=-1.75, p=0.006) and positively associated with feeding frequency (*b*= 1.24, p< 0.001) and energy density (*b*=31.29, p<0.001).

Table 4.14: Multiple linear regression coefficients of determinants of energy intake by child body weight (kcal/kg) of children <36 months of age during one 6-hour observational period in low-income peri-urban communities of Lima, Peru, by pregnancy status

Variable	Standardized coefficient[1]	SE	P value
Early pregnancy	0.163	3.04	0.08
Late pregnancy	-0.004	3.07	0.96
Feeding frequency (feeds/6-hour)	0.335	0.32	0.0002
Energy density (kcal/g)	0.442	5.99	<0.0001
Household members (#)	-0.257	0.62	0.006
Owned residence	-0.224	2.78	0.02

[1]Model: n=89, adjusted r^2= 039, SE= 11.7, p<0.0001

5 DISCUSSION

There is limited information available about the lactation-pregnancy overlap, and even less on its effects on the dietary intake of a breastfeeding child. The results from this study add to the dearth of information available by demonstrating a decrease in breast milk intake but no overall difference in total energy intake in the children of pregnant mothers, however it is important to note that this study had a lower than expected enrollment. Despite the limitation of low enrollment numbers, this study had a rigorous research plan and recruitment strategy in addition to thorough pre-testing of all data collection tools and standardization with all fieldworkers.

It may be that with a larger sample size other associations may have been found. In this study, due to the small size and inherent errors of dietary methods, it is possible that we observed unusually lower breast milk intake in the non-pregnant group than what is common of breastfeeding children of the study age in developing countries. However some of the data presented in this population had little variance, such as low food insecurity and civil status, and would most likely not change with a greater sample size. Additionally, it would have been expected that some data influenced the outcome variable but were not seen to have an association (i.e., child's age on total energy intake), most likely because of the small sample size. Therefore, the observations described below are limited by this small sample size but still provide valuable insight into several issues from which future research may benefit.

5.1 Children's breast milk intake

A reduction in breast milk intake in both the LP and EP groups could be the result of many factors. First, it could be the reduction of child demand. It has been seen in animal research that milk composition changes during pregnancy and before parturition, with the milk containing more protein and less lactose and fat[8]. In humans, this could change a child's feeding patterns as it may alter the mouthfeel or taste of breast milk, and therefore the desire of the child to breastfeed. In addition, it has been seen in animal studies that milk production declines during pregnancy. If this is also the case in humans, a child may suckle but be less successful at extracting the milk[8]. Therefore, with decreased quantity and change in composition, children may change their feeding patterns. One study examining the changing composition of human milk in addition to the development of an appetite control, found that as long as breast milk was still available for the child, it may be a change in taste or texture of the breast milk that affects infants desire to breastfeed[113]. The researchers concluded that change in milk composition is a cue for a child to stop feeding. Other studies have found that change in breast milk taste affects the children's breastfeeding practices as well. One study found that mothers on drug therapy had feeding difficulties that may have been attributed to a change in breast milk taste[114]. Another study found that children had a decreased acceptance of post-exercise milk because of the change of taste due to an increase in lactic acid concentration[115]. However, additional studies are needed on the relationship between change in milk composition and child's feeding patterns.

Another reason for decreased breast milk intake may be changes in maternal feeding behaviours. Pregnant mothers may perceive that their children do not consume a sufficient amount of breast milk and therefore may attempt to increase milk intake by breastfeeding more frequently or by increasing other non-breast milk substitutes. In our study, the LP and EP groups had fewer breastfeeds than the NP group so it is more likely that the mother changed her feeding practices and provided other substitutes than trying to increase her breastfeeding frequency, however this study did not explore the cultural reasons for these changes.

Lastly, a child can have a decreased intake of breast milk because the mother has limited milk production. Insight can be drawn from animal models, as lactation during pregnancy has been shown to have negative effects on milk production in animals. Olori and colleagues found that daily milk yield in cows declined due to pregnancy between month one of gestation until parturition[8].

Significant decreases in milk yield started in the fifth month of pregnancy and became greater further along in the pregnancy. When the cows were in the eighth month of pregnancy, daily milk yields were about 11% below the production level of non-pregnant cows. The mechanism of reduced milk production in cows during pregnancy is not well understood. It may be that during pregnancy there are elevated levels of estrogen and progesterone, which inhibit prolactin, the hormone necessary for milk production and for stimulation of the mammary glands. It is possible these hormonal influences decrease milk production but these mechanisms are not well understood in humans. It is not known if physiologically the body is able to adapt to these hormonal influences. Milk production may also be reduced when maternal diet is extremely limited but it is unlikely that the women in the present study had a limited diet as evidenced by their body mass index. Marginal limitations in maternal diets have not been seen to have an effect on milk production[71, 116, 117].

Not only was pregnancy status associated with breast milk intake in our study but it was also associated with maternal age and education. Maternal age was negatively associated with breast milk intake in this study. One study examining the determinants of breastfeeding in Sri Lanka found that as mothers aged they changed their breastfeeding practices and were more likely to wean their child at an earlier age[118]. It may be that in the present study as mothers aged they changed their breastfeeding patterns and decreased either frequency or time at the breast, which would limit the child's intake of breast milk. However, there is no evidence that milk production decreases with age[119].

Results are mixed in the literature with regard to the association between maternal education and breastfeeding practices. One study in Bangladesh found that mothers with higher education were more likely to wean their child at an earlier age and their children spent significantly less time at the breast than mothers with less education[59]. Conversely, one study in Ghana found that poor maternal schooling was the main constraint to child feeding practices, including introduction of complementary foods before six months, providing inappropriate complementary foods, and poor responsive feeding[48, 120]. Educated mothers in low-income countries have been observed to work away from the home more often than less-educated mothers and therefore, tend to breastfeed less frequently or may wean their child earlier[59, 118]. In the present study, mothers' employment was not associated with breast milk intake. However, mothers who were employed were more likely to have a high school education than the mothers who were not employed, although not a significant difference (63% vs. 53%, respectively; p= 0.17).

In addition to these predictors of breast milk intake, child characteristics such as age, sex, weight, and length/height, and other household socio-economic status variables such as household assets, crowding, and money spend on food, were tested in the model but were not found to be significant. It would be expected that child age would affect breast milk intake as it is well known that as children age, their consumption of complementary food increases and provides the majority of their total energy intake. In this study, child age was not associated with breast milk intake but a negative trend in the data was seen and with a larger sample size, may have been significant and therefore, included in the model. We found that breast milk consumption was low, contributing very little of the total energy intake, thus the results might be different in a context with a higher proportion of energy from breast milk.

5.2 Children's total energy intake

An increase in total energy intake observed in the EP group could be due to various reasons. One possible explanation is that the mother was able to perceive that her child's breast milk intake had decreased or she perceived that she was not producing adequate breast milk and therefore supplemented her child with more complementary food.

"Insufficient milk syndrome" was termed in the 1980's and now more recent studies of milk insufficiency have concluded that cultural practices, psychosocial factors, and breastfeeding behaviour are directly linked to perceived milk insufficiency[74, 121]. WHO has recognized that insufficient milk production is a public health concern with children under 6 months since only 35% are being exclusively breastfed[122]. Although, insufficient milk syndrome is most often experienced during the period of exclusive breastfeeding, it may be also be experienced at later times.

Behavioural studies in the developing world have indicated that pregnancy is a common reason for mothers to change breastfeeding practices. One study in Bangladesh found that, out of the 1419 women who had delivered a live birth during the study period, 18% stopped breastfeeding because of perceived insufficient milk production[59]. Of the mothers who discontinued breastfeeding because of a perception of having insufficient milk, 59% of them had become pregnant. Therefore, it may be that a mother discontinues breastfeeding because of her new pregnancy instead of, or in addition to, her perceived insufficient breast milk production.

Another reason could be that with the new pregnancy the mother's perception of her child's age may have changed. Until the new pregnancy, she may have treated her young child more as an infant and continued to provide more soups and liquids. With the new pregnancy, she may now think of her child as older, since he/she will soon be the older sibling to a newborn, and therefore, begin to provide foods more appropriate for the whole family. One study conducted in Peruvian toddlers found that differences in feeding patterns between breastfed and non-breastfed toddlers may be due to maternal perceptions of breastfed children as "still young" and therefore the mother continues to feed the child as an infant rather than as a young child[123]. Similarly, it is possible that in the present study the mother may have thought of her breastfed child as "still young" but instead of it being a weaned child that changed the mother's perception, it was her pregnancy status. This change in mothers' perception of her child's age has not been well studied and more information is required on how it affects children's dietary intake.

Not only was pregnancy status associated with total energy intake in the present study, energy density and feeding frequency were found to have a positive association with total energy intake. Brown et al. conducted a study to evaluate energy density and found that, overall, the energy intake of Peruvian young children was more than twice as great with the highest energy density foods compared with the lowest[37]. Their study demonstrated that when energy density was taken into account, the total daily consumption was about 16% greater when the number of meals was increased from three to four per day. In our present study, when energy density increased by 1.0 kcal/g, total energy intake increased by 373.6 kcal/d. Similarly, in the study by Brown et al., when energy density increased by 1.1 kcal/g, total energy intake increased by approximately 400 kcal/d. Since feeding frequency was defined differently in the present study, it cannot be directly compared to the study by Brown et al.; however, increases in feedings in both studies were positively associated with energy intake.

In addition to these predictors of total energy intake, child characteristics such as age, sex, and length/height were tested in the model but were not found to be significant. This may be partly explained by the dependent variable being energy intake per kilogram of child body weight, therefore accounting for weight variations in the children. Socio-economic status variables were also tested in the model but were not found to be significant, as explained earlier there was little variation in the socio-economic variables in this peri-urban population, and the variables used may not have captured actual differences.

5.3 Child nutritional status

The percentage of children in the present study who were classified as underweight, wasted, and stunted was lower than the national percentages reported by the Peru Demographic and Health Survey in 2009[98]. However, the prevalence of stunting in our study (18.5%) was higher than the Lima regional prevalences (8.6%), while the prevalences of wasting and underweight were similar. These compared prevalences to the national and regional levels may be explained by our study being conducted in peri-urban communities outside of Lima with a population that is primarily urban yet includes several recent rural migrants. It has been shown that children from urban areas have less stunting than rural areas, which may explain why the prevalence of stunting in our study was lower than the national level, which includes several rural regions, yet higher than the Lima region since includes a dominant urban community. The similar wasting and underweight prevalences to the Lima region may be explained by i) the rates are low and therefore, not likely to find significant differences and ii) because children in these peri-urban communities are not at a risk of low energy in their diet that cause underweight, instead it is the poor quality of their diet that leads them to be stunted.

In our study, there was no significant difference between a child's nutritional status and a mother's pregnancy status, although in bivariate analysis HAZ approached significance. When HAZ was adjusted for confounding variables, there was no significant relationship between pregnancy status and HAZ, although there were associations with total energy intake and with total household assets. In univariate analysis pregnancy status was a predictor of HAZ. There were some group differences in socio-economical status between the groups food expenditure was lowest in the LP group followed by the NP group and then the EP group, ownership of residence was highest in the LP and EP groups, and number of material household assets and houses with permanent wall structures were lowest in the LP and EP groups. However, no other socio-economic variable besides household was a significant predictor or HAZ and pregnancy status was no longer significant when household material assets. Some studies have found that children from households with lower economic wealth were more likely to suffer from stunting compared to households with higher economic wealth[52, 53]. One case-control study in Mexico found that *per capita* monthly family income was not significantly associated with child nutritional status in rural areas but was positively associated in urban areas[124]. All households in our study were economically disadvantaged but were generally less economically disadvantaged than the households in the Mexican study on economic wealth. This is most likely due to the fact that these studies on economic wealth were primarily in rural areas, with the exception of part of the study conducted in Mexico, which was comparing urban and rural areas. Our study was conducted in a peri-urban area and it is well known that urban areas have higher socioeconomic status than rural areas[125]. Household size, proportion of households with a dirt floor in their home, and the proportion of mothers working were the same in our studies and the studies examining economic wealth but there were more households in the present study with indoor plumbing and a sewage system than the urban households in the study conducted in Mexico. The present study, therefore, had similar findings to other studies conducted in both rural and urban areas, and confirmed that socioeconomic status is positively associated with HAZ.

In agreement with previous reports, total energy intake was also significantly positively associated with HAZ. It is well known that an inadequate energy intake is a cause of growth faltering. Other reports have been conducted to see the effect of different energy levels on children's growth and have seen that children whose diets were supplemented with high-energy foods showed both increases in weight and height[37, 38].

5.4 Methodological challenges and limitations for the analysis of the early pregnancy and non-pregnant groups

5.4.1 Study Design

To address the research objective, a cross-sectional study design was planned. The study population included women over 18 years of age and of reproductive age with a child under 3 years of age who was still breastfeeding at the time of enrollment. The sample was randomly selected from an ongoing community census in the study site, however. The study site was chosen because of its known prevalence of the pregnancy/breastfeeding overlap, along with resources provided by the IIN. The IIN has had a permanent research presence in these peri-urban shantytowns since the 1980's and their field center provided a good base for data collection. With the availability of transport vehicles, nutritionists, Spanish speaking experienced fieldworkers, equipment, and communities familiar with the IIN, data collection was made feasible in this study site. The study design consisted of one in-home six-hour observation and one 18-hour dietary recall to attain a 24-hour period with dietary information. Maternal consent was obtained two days prior to the observation, which allowed the transport vehicle and the fieldworkers to become familiar of the location of the house. This study design was feasible for this research project, however, it limited the information that could be a collected. A longitudinal study following the mother from the first trimester of pregnancy until parturition collecting data with multiple 24-hour observations would have provided a better understanding of maternal physiological adaptations to the pregnancy-lactation overlap and maternal feeding practices during the overlap period.

Another limitation to this study design is that the mother chose whether she was in the NP or EP group; they were not randomly assigned to continue breastfeeding during their pregnancy. These mothers were part of a group because of her choice in infant feeding behaviours.

5.4.2 Twenty-four hour dietary information

The dietary observation method is regarded as the most accurate of all methods of dietary field survey methods, since there is the precision of actually weighing the foods and liquids consumed, and is considered the "gold" standard[126]. It is, therefore, used frequently as the reference method when validation for more advanced and extensive studies is needed. This study conducted a 6-hour observation and therefore was able to obtain accurate measurements for only part of the day. In Peru, the lunch meal is when people consume the most energy, so an observation during this period was advantageous, however, a 24-hour observation period would have permitted a better estimate of full-day energy consumption. Another limitation to the 6-hour observation was that it was not possible to measure breast milk intake for all children since not all mothers breastfed during the lunch period; it was more common for the mothers in this study to breastfeed at night.

Due to the invasiveness of dietary observations in the homes and lives of the families, this method may have produced changes in the child's eating and breastfeeding habits. It may not have been solely that mothers breastfeed more often at night that measuring breast milk for all children during the observation period was not possible; mothers may not have felt comfortable with the fieldworker present. Additionally, mothers knew that this was a study examining determinants that influence their child's dietary intake, so mothers may have been more inclined to force the child to eat more during the observation or provide the child with healthier food options such as fruit. Therefore, there is a trade-off between the precision and accuracy of this method and the risk of eliciting bias in the results. Previous studies in a similar community in Lima showed that there was minimal to no change in the diet of children in the presence of a fieldworker, in part because of limited economic possibilities for variation[127]. The fieldworkers were also chosen from similar communities and trained not to interfere with or comment on the foods that were prepared or served to the children.

The dietary recall method relies on the mother's ability to remember all foods, liquids, and breast milk consumed by the child. Since memory of food consumption declines rapidly beyond one day, the dietary recall method was always conducted the day immediately after the observation. Although all precautions and steps necessary were taken to receive the most accurate information during the dietary recall, there was still a chance of foods being missed or the portions of foods being misreported. It was very common in this study population for the mother to say that the child finished eating all food items and meals provided outside of the observation hours; however, very rarely did the child finish a full meal during the observation period.

Ideally one 24-hour dietary record/recall would have been conducted for each child each month for three months as is recommended in Willet's book *Nutritional Epidemiology* [128] but due to time constraints and limited resources, only one 24-hour period was documented. This creates difficulty in determining if this one 24-hour period was an actual representation of the usual dietary intake of the child. Although bias could have resulted from both the observation and the recall, when consent was obtained to participate in the study, mothers were well informed that the observation was intended to be a "normal" day for the household and a representation of what the child typically consumed. Since the study's objectives were to determine the association of pregnancy status on child's dietary intake, food frequency questionnaires and dietary history methods would have been less effective energy intake survey methods.

5.4.3 Breast milk intake estimates

There are many different methods of estimating breast milk intake; the problem is that the techniques for measuring breast milk are limited by because the impact of the study procedures and methods on lactation estimates remains uncertain. Brown et al. concluded that the procedures that are the least invasive and that require the minimum changes from the natural setting are most likely to get the closest estimate of a normal breastfeed[64]. However, the issues of cost, comfort of study participants, and the level of technical sophistication need all must be considered in planning for the study. This study chose test weighing of the child accounting for insensible water loss. Weighing the child before and after the breastfeed has been assumed to equal the weight of milk consumed and is the most commonly used technique[64]. The procedure is technically simple and requires little field training and equipment and does not interfere drastically with usual breastfeeding. However, sometimes the milk consumed at one time may be small and therefore, the measurement error can introduce variability that is a larger proportion of the amount consumed. The test weighing method also consistently underestimates the amount of milk consumed, which may be due to small amounts of milk that were spilled or regurgitated[64]. Additionally, if any urine or stool passes after the initial weighing and is not included in the final weighing, this would change the estimate.

Our study used a balance accurate to the nearest gram, weighed the child with the same clothes before and after, and had field workers who were at ease with the procedures to minimize variability. Had we taken milk samples we would have been able to measure the quality of the breast milk including energy, fat, and protein, however due to limited resources, this was not an option.

5.4.4 Breast milk extrapolation

The test-weighing procedure of measuring breast milk intake was chosen as the most accurate method available for this study since it was feasible for implementation in the field during the 6-hours observation period. It would have been possible to have only analyzed the 6-hour observation but a 24-hour period provides a better idea of feeding practices. Unobserved breast milk intake was estimated from the number of feeds recalled by the mother and multiplied by the average intake per observed feed in the study. Therefore, this study relied on mother's recall for her breastfeeding frequency. Maternal recall for breastfeeding practices has been examined in other studies and has been found to be a reliable source, especially when conducted the following day[129-132]. Studies have shown that the longer the period between the day of interest of dietary intake and the dietary recall, the poorer the reliability[132]; in this study, every dietary recall was conducted the following day and normally in the morning. This method, although not error-free, was considered the best approach for data extrapolation. Ideally, 24-hour test-weighing procedures of measuring breast milk intake would have been conducted for all children, or having measured breast milk intake for five to ten children would have permitted regression coefficients to be calculated to translate for the 6-hour breast milk consumption to 24-hour intake. Twenty-four hour breast milk measurements were identified in 36 children of similar age in the same community but due to the mother being in her third trimester of pregnancy, breast milk intakes were negative and therefore not representative of the breast milk intakes of the children in the present study (Marquis et al, unpublished data). Since 24-hour measured intake information was available from these unpublished data, an estimate of the correlation between the 6 and 24-hour data was able to serve as an indication of the potential error incurred from the partial (i.e., 6-hour) data. The total mean breast milk intakes only varied by 1.5 grams, which provides some validity to the approach used.

5.4.5 Recruitment and sample size

The children enrolled in this study were all part of similar peri-urban communities found from the community censuses. Since these study communities are considered “pueblos jóvenes” or young towns outside of Lima, the government does not have a record of the population or maps of the areas. These areas continue to expand further into the hills and houses are being built behind and on top of other houses making it challenging for the fieldworkers to know exactly where each house was located or if new houses had been built, which may have resulted in missed or inaccurate information.

The community census used for this study only identified 49 breastfed children whose mother was in her 1st or 2nd trimester of pregnancy. If it had been possible to expand the census to other communities, to revisit the same communities, or to continue the census for a longer period of time, it may have been possible to reach the desired sample size. A larger study sample would have increased statistical power, therefore, would have provided more definitive results. Unfortunately, due to time and resource constraints, further recruitment was not an option.

The original sample size was thought to be feasible since past studies had reported that in Canto Grande the annual birth cohort had been about 10,000 for many years and that 40% of all pregnant women had a child less than four years of age. Additionally, in a previous study, it was found that 60% of women continued to breastfeed in their first and second trimesters of pregnancy (of a new pregnancy). Since the IIN has been well established in this community, and other studies had low refusal rates, enrolling 79 pregnant and lactating women seemed reasonable. However, the community census only identified 49 mothers in early pregnancy who were also breastfeeding their child. Possible reasons for this low number were 1) a lower than expected birth rate seen in the study area, 2) an increase in the number of women working and therefore not at home during the census, and 3) messages from the local health centers discouraging mothers from breastfeeding once they are newly pregnant.

Forty-two mothers refused to participate, which was more than expected but this could be due, again, to increased employment among women. It was sometimes hard to contact a working mother even when she had been identified by the census as a pregnant and lactating mother since she was normally at her work during the home visits of the fieldworkers to discuss the study. Another reason for refusal was that some families did not feel comfortable inviting a fieldworker into their home. Multiple families might have been present in the same house and resulting crowding might not have been conducive to participation in the study. All precautions were taken to minimize any real or perceived discomfort that might have been experienced by mothers and families but in some cases these precautions were not sufficient.

There was a higher refusal rate in the NP group compared to the EP group. This is most likely because the pregnant mothers were revisited up to three times to discuss the project. Once we had visited three times, it was considered a refusal. On the other hand, since there were more NP mothers to choose from, and to not waste time on enrollment, they were only visited once and if they were not interested, on the first visit it was considered a refusal. Therefore, it is possible that this enrollment process could have led to selection bias; however, it is unlikely that this enrollment method led to a difference in the study participants since most of the maternal and household characteristics were not significantly different.

5.5 Limitations for the addition of the late pregnancy group

Since the NP and EP groups had a 6-hour observation and 18-hour recall and the LP group had a 24-hour observation, only the comparable 6-hours were used in this analysis. It is possible that since the EP and NP mothers knew that the fieldworker was with them for only 6-hours, she refrained from breastfeeding or providing certain foods until after the fieldworker left, while in the LP group, the mother knew that the fieldworker was present for the full 24-hours and therefore, did not have the same mentality of waiting until she was alone and breastfed more frequently or provided the usual amount of food during the extracted 6-hour observation. As stated previously, mothers were well informed that the observation was supposed to be a “normal” day for the household.

6 FINAL CONCLUSIONS

Few researchers have looked at the consequences of a lactation-pregnancy overlap, and those who have researched this overlap have only examined the health consequences for the newborn and the mother. To our knowledge, this is the first study to document associations between the practice of breastfeeding during pregnancy and the dietary intake of infants and young children. In this study, the overlap of breastfeeding and pregnancy was found to be associated with changes in breast milk but not with total energy intake. Regardless of the mother's decision to continue breastfeeding during pregnancy, these results demonstrate that complementary foods make up the vast majority of the diet. Overall, these results provide new insights into determinants of Peruvian infants and young children's diets and health and can be used as a stepping-stone for future research and nutrition education. This study demonstrated that mothers are able to adapt their feeding practices in certain conditions to maintain an adequate energy intake for their child. Given the low intake of breast milk, nutrition education should focus on assuring the quality of these complementary foods as these findings suggest that for these age groups in these communities, children depend on complementary foods to provide the essential nutrients and energy for growth and development.

6.1 **Future research**

The present study was observational and cross-sectional in design. Longitudinal studies following the mother from the first trimester of pregnancy until parturition would provide a better understanding of maternal physiological adaptations and child feeding practices during the pregnancy-lactation overlap period. More research is needed on the mechanisms of breast milk production during pregnancy and to examine maternal and child adaptations. Additional research is needed to assess the possible short-term and long-term implications on maternal, child, and newborn health.

This study was conducted in a peri-urban community in Peru with low food insecurity, future research would need to be conducted to understand the implications of early and late pregnancy of breastfeeding children in other cultures and contexts. Future studies could also include a qualitative component to better understand where the mothers receive most of their information regarding breastfeeding practices, which may provide interesting new avenues of investigation and provide a better understanding of breastfeeding practices.

6.2 Relevance and recommendations

It has been demonstrated that the pregnancy-lactation overlap is still practiced among women in peri-urban communities of Lima. Therefore, it is essential that healthcare professionals in these communities, and anywhere else where this overlap is being practiced, be given evidence-based nutrition information to share with women who choose to continue breastfeeding during their pregnancy. Stunting and malnutrition are common in infants and young children and therefore health care professionals should be provided with information on the pregnancy-breastfeeding overlap, along with information for other determinants that affect dietary intake, as adequate dietary intake is essential for growth and development during this critical period.

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8 APPENDICES

Appendix 8.1: Informed Consent Form

Study Title: Influences of Toddlers Dietary Intake

McGill University, Montreal, Canada:
Allison Verney, MSc candidate, School of Dietetics and Human Nutrition
Grace Marquis, Ph.D., School of Dietetics and Human Nutrition
Theresa Gyorkos, Ph.D., Dept. of Epidemiology, Biostatistics, and Occupational Health

Instituto de Investigación Nutricional, Lima, Peru:
Av. La Molina 1885~ La Molina, (51-1) 3496023
Mary Penny, MD
Rossina Pareja, MSc

Introduction
The Instituto de Investigación Nutricional is an institution that has been working in Peru for over 50 years conducting projects to improve the health and nutrition of mothers and their children. This is a project being conducted in collaboration with the institute and we invited you to take part in it. Through this consent form, we would like you to understand exactly what we are doing and what being a part of the research means to you. We ask for you to please read this consent form, take your time in deciding if you would like to participate, and if so, we would ask you to sign the form. If you have any questions or need any clarifications, please feel free to ask us. After, we will give you a copy of this form.

What is the purpose of this study?
The purpose of this study is to examine the dietary intakes of toddlers and what influences their intake.

Who can participate in this study?
Women over 18 years-old who have a child between the ages of 12- 48 months and who are breastfeeding this toddler.

What is my part in the research?
If you agree to participate in this study, your participation will involve two visits in your home. The first visit will be to observe all the food, liquids an breast milk your child consumes; in this visit our fieldworker will arrive to your home between 8 and 10 am and will stay for up to six hours. In this period the fieldworker will weigh all the foods used to prepare lunch and snacks, and weigh your child before and after every breastfeed. Also, there will be a questionnaire where we will be asking about yourself and your family members, among other topics. During the visit we will also weigh and measure your child. The day after

the observation the fieldworker will return to ask you about the foods the child ate after the observation of the previous day, this will take about 45 minutes.

What risks are there if my toddler and I participate in this study?
There are no risks in participating in this study since we will obtain all the necessary information through your responses to a questionnaire and through weighing your toddler and the food administered to your toddler. Sometimes families may feel uncomfortable by having someone in their home but we will try our best to inconvenience you as little as possible.

What benefits are there for our participation?
There will be no direct benefit for you, however, the valuable information gained from your participation will benefit society by providing necessary knowledge and insight on factors that affect toddler's dietary intake. This information may be used to develop appropriate feeding practices in the future.

If I participate, what are my rights?
Your participation in this study is completely voluntary and if at any moment you feel uncomfortable or simply do not wish to continue, you have full right not to participate and withdraw from the study at any time. If you leave the study or refuse to participate there will be no consequences to your health services or your ability to participate in other studies.

Will this be done in confidentiality?
All records that would identify you or your toddler or that contain information about you or your toddler will be kept confidential and will not be made publicly available. To ensure confidentiality, you will be given a unique number code, and this code will be used on forms instead of your actual name. Only the study's researchers will have access to the records that link your code and your name. Documents will be safely stored at the institute and all electronic computer files will be password protected. Upon completion of this study, all files linking your code and your name will be destroyed. If the results are published, your identity will remain confidential. Any photographs taken of you or your child, with your approval, will not have your name, your toddler's name or your community associated with it.

Who can I contact if I have any questions or concerns about this study?
You are encouraged to ask any questions at any time during this study. For more information about this research, please contact: Allison Verney +1-438-883-9770 allison.verney@mail.mcgill.ca (Canada); Dr. Grace Marquis +1-514-398-7839 grace.marquis@mcgill.ca; (Canada) Dr. Mary Penny 3496023 mpenny@iin.sld.pe (Peru) or Rossina Pareja 3496023 rpareja@iin.sld.pe (Peru). You may also wish to contact the Instituto de Investigación Nutricional's ethics committee, Dr. Hilary Creed-Kanashiro, for any questions regarding the rights of research subjects or the REB McGill contact, Dr. Blaine Ditto +1-514-398-6097.

Voluntary statement of informed consent form
Your signature indicates that you voluntarily agree to participate in this study and that the above document describing the benefits, risks and procedures for the research study has been read and explained to you, you have been given adequate time to read the document, and that your questions have been answered to your satisfaction.

I agree to participate.

____

Participant's Name (Printed) Date

____

Participant's Signature

Appendix 8.2

INTAKE OF FOODS AND LIQUIDS OTHER THAN BREAST MILK

Code

Date
DD MM YY

Visit #

Page # of

1	Breakfast
2	Lunch
3	Dinner
4	Snack

CODIGOS DE FORMA DE PRESENTACION

1	pure water	9	sugary drink	17	thick puré	25	Fruit and vegetables whole/piec
2	water with sugar	10	soft drink	18	pudding without milk	26	bread/cookies/cake
3	Infusion with sugar	11	Suero	19	pudding with milk	27	sweets, ice cream, candies
4	Infusion without sugar	12	Caldos	20	boiled and mashed foods	28	Ham/cheese/tuna
5	milk, yogurt	13	soup	21	boiled whole foods (potato, sweet potato, egg)	29	Entrée (potatoes with cream, cev
6	cereal with milk	14	thick soup	22	cooked starch (rice, wheat, pasta)	77	others, specify
7	cereal without milk	15	cream soup	23	Mixed stews(stews/ stir fry) GUISO???		
8	juice: fruit or vegetable	16	liquified pur	24	Fried food (fish, meat, egg, chicken, potato)		

1	Observación
2	Recordatorio

Order #	Type of meal	Observ. /record.	Forma de Presentación	Start time	Recipe	Código	Ingredient	Code	FINAL/COOKED SERVED FOOD 1. Container (g)	2. Container & prepared/cooked food	Served prepared/ cooked food(=2-	Desperdicio o sobras Container (g)	Tara + desperdicio ó sobras (g)	Desperdicio ó sobras (g)	End Time

OBSERVACIONES:

Appendix 8.3

RECIPE PREPARARTION

CHILD-MOTHER: ____________________

RECIPE NAME ____________________

Code | | | | |

RECIPE CODE

PLACE OF PREPARATION

1:Observation, 2:Recall

01	House	05	Restaurant
02	Comedor*	06	Food fom previous day
03	Neighbour/family	07	Other, specify
04	Market	99	No Information

Date DD MM YY

Page # ___ of ___

N° Order	Food	Code: Recipe	Code: Food	Serving plate (g)	Serving plate + food (g)	Weight of food (g)

Total Weight of the Cooked Recipe		
Weight of Pan (g)	Pan+ cooked recipe (g)	Cooked recipe (g)

Beginning time of preparation	:
Final time of preparation	:

OBSERVATIONS: ____________________

*A place where you can purchase a set menu, normally from someone's home

Appendix 8.4

DATE | | | | | | |
DD MM YY

INTAKE OF BREAST MILK

Visit # [ ]

Code [ ] [ ] [ ] [ ]

Hours of Observations: Began:_ _:_ _ Ended:_ _:_ _ Total # of hours observed:_ _:_ _

Page # [ ] of [ ]

	Number of Breastfeeds				
WEIGHTS	1	2	3	4	5
Weight before breastfeed (g)					
Weight after breastfeed (g)					
Total weight of feed (g)					

HOURS	TIME OF FEEDS				
Hour began feed (use military time)	:	:	:	:	:
Hour finished feed (use military time)	:	:	:	:	:
Duration of feed					
total minutes on breast that didn't fee					
Total minutes breastfed					

Observations ____

8.4

Appendix 8.5

Date ☐☐☐
DD MM YY

Socioeconomic and Maternal Information

Code ☐☐☐☐

Maternal Records

1. Where were you born? ______________ Department
   ______________ Province

   1a. In which zone? 1.costal 2. mountains 3.jungle ☐

2. Which language did you learn to speak first? ☐☐
   1. Spanish 3. Aymara 77. Other ______________
   2. Quechua 4. Indigenous language

3. Mother's age: ☐☐ years 4. Birth date ☐☐☐
   DD MM YY

5. How long have you lived in Lima (total)? ☐☐ years

6. How long have you lived in Canto Grande? ☐☐ years

7. Do you have family who live close in Lima? ☐ 1=yes 2=no
   parents, brothers, uncles, aunts, cousins

8. Do you have family who live close in Canto Grande? ☐ 1=yes 2=no
   parents, brothers, uncles, aunts, cousins

9. ¿Until what year did you study? ____ ____ ☐
   0. preschool
   1. incomplete primary
   2. complete primary
   3. imcomplete secondary
   4. complete secondary
   5. college incomplete
   6. college completed
   7. universary imcomplete
   8. university complete

10. ¿What is your marriage status? ☐
    1. married/ common-law
    2. single
    3. seperated
    4. divorced
    5. widowed

11. Do you currently work? ☐ 1=yes 2=no
    (if no, go to q.12)

    11a. Type of work (occupation) ______________ ☐☐ see code list

12. Have you worked this past year? ☐ 1=yes 2=no
    (if yes, go to q. 13)

code

12a. How long has it been since you stopped working? months

13. Do you have health insurance? 1=yes 2=no 3=trying to get it
(if no, go to q. 14)

13a. What type?

1. EsSalud 2. Particular 3. SIS 4. seguro MINSA 77. other ____________________

14. Who is the principle provider of your house?

1. husband or partner
2. mother/father
3. mother/father-in-law
4. herself
5. other family member
77. other__________

15. What is the occupation of the principle provider?

15a. occupation type: ______________________ see code list

LIVING

16. The house you ive in is:

1. yours
2. rented
3. family members
4. guarding it
77. other ____________________

17. Observe and note the material that the house is made of:

1. finished brick
2. unfinished brick
3. cardboard
4. plywood
77. other ____________________

18. Observe and note the material that the floor is made of:

1. wood/ceramic
2. finished cement
3. unfinished cement
4. earth
77. other ____________________

19. What do you use to light you ______________

20. Where do you get your water for the house? ______________

21. Where do you do your necessities (c ______________

22. What type of fuel do you use for your house?

23. What type of electronics do you have in your house? (that work)

		1=yes 2=no	How many?
1.	refrigerator		
2.	blender		
3.	television		
4.	radio		
5.	computer		

		1=yes 2=nc	Cuántos
6.	sound system		
7.	oven		
8.	washing machine		
9.	sewing machine		

code

24. How many family members are in your house?
(who sleep in the same house)

children 0-5 y. | children 6-11 y. | Adolescents 12 - 17 y. | Female adults >18 y. | male adults >18 y.

25. How many rooms are in your house?
excluding bathroom and kitchen

25a. How many are used for sleeping?

26. Do you sleep in the same house that you eat? 1=yes 2=no

27. Where do you spend the majority of the day? 1=your house, 2=other house

28. If it is another house, what is your relationship with the owners?
1. parents
2. in-laws
3. siblings/siblings-in-law
4. other family members
5. friends/ neighbours
77. other ______________

29. How many people share meals together?

children 0-12 y. | adolescents 12 - 17y. | Adults >18 y.

30. How many people share food expenses?

31. How much money does your family spend on food a day?

32. DO you get food from places outside the house? 1=yes 2=no

32a. From where?

Place	1=yes 2=no	Frecuency times/month
Restaurant		
Glass fo Milk		
NGO		
other		

code

YOUNG CHILD

33. age of child ____ months birth date ____ (DD MM YY)

34 Sex: ____ 1=M, 2= F

35. birth weight ____ g. 36. birth length ____ . ____ cm.

37. Did they show you there CRED control card with information? ____
    1. Yes 3. Yes, only with weight
    2. No 4. Yes, only with height

38. Was your child premature? ____ 1=yes 2=no

39 In the last week, how often each day have you breastfed? day ____ night ____ total ____

PREGNANCY INFORMATION

40. Have you had an ultrasound? ____ 1=yes 2=no

40a. ¿Did they show you your ultrasound? ____ 1=yes 2=no

40b. Date of ultrasound ____ (DD MM YY)

40c. Suggested gestational age from ultrasound ____ weeks ____ +/- days

41. Do you know the date of your last menstrual period (LMP)? ____ 1=yes 2=no

41a. LMP ____ (DD MM YY) 41b. Are you sure? ____ 1=yes 2=no

42. Do you smoke? ____ 1=yes 2=no

43. If not, have you ever smoked? ____ 1=yes 2=no

43a. How long has it been since you've quit smoking? ____
    1. Less than 6 months
    2. More than 6 months

Appendix 8.6: Socioeconomic Status Codes

Type of work: question 11a. y 15a.

1. Services (carpenter, shoe maker, gardener)
2. Construction
3. Sales
4. Multiple jobs
5. Transportation
6. Home cleaner and laundry washer
7. Security
8. Professional
9. Obrero

77. Other
88. Do not know

Type of electricity: Question 19

1. Connected home electricity
2. Pulled electricity from neighbour
3. Lantern
4. Candle

77. Other

Water source: Question 20

1. Potable water in the house
2. Public sink
3. Tanker
4. Parents/ neighbours
5. Well

77. Other

Place used as a bathroom/ necessities: Question 21

1. Toilet with lid and connected sewage
2. Bacin
3. Outhouse with drainage
4. Outhouse without drainage
5. Shared outhouse
6. Borrowed outhouse
7. Open land
8. Shared toilet

77. Other

Fuel used for cooking: Question 22

1. Gas
2. Kerosene
3. Electricity
4. Wood
5. No kitchen

77. Other

Appendix 8.7

Code | | | |

Toddler's Anthropometrics

visit # | | Date | | | (DD MM YY)

Sex | | 1=boy 2=girl

Date of Birth | | | (DD MM YY)

Interviewer code | | |

Months old | | |

Weight | | | . | kg.

Height | | | | . | cm

Observations